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VOLUME 21 NUMBER 8

AUGUST, 1931

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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An International Journal
Official Organ of The American Phytopathological Society

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PHYTOPATHOLOGY

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AUGUST, 1931

SPORE GERMINATION OF PUCCINIA GLUMARUM WITH NOTES ON RELATED SPECIES[1]

J. M. RAEDER AND W. M. BEVER[2]

INTRODUCTION

According to Humphrey, Hungerford, and Johnson (13), stripe rust, *Puccinia glumarum* (Schm.) Eriks. and Henn., was first described in Europe by Schmidt (24). He, apparently, was cognizant only of the uredinial stage, for, in describing the fungus, he named it *Uredo glumarum*. Eriksson and Henning (6), in 1894, having discovered the telial stage, reclassified the organism and named it *Puccinia glumarum*.

Believing the non-existence of stripe rust in the United States east of the 103rd meridian might be explained on a basis of specific and inherent sensitiveness of the organism to environmental factors rather than its host relationships, the writers have sought to determine the effect of a partially controlled environment on the longevity and germination of both urediniospores and teliospores. It seemed important to know more concerning their viability and longevity under controlled conditions, not only as an effort to explain the absence of stripe rust in the Central and Eastern States, but also because of the application of the results to future research on this rust. A study of the germination of the teliospores was undertaken primarily to ascertain the optimum conditions for their germination and the development of the sporidia. This phase of the investigation was deemed preliminary and necessary to the proper carrying out of any organized investigation of the relation of *Puccinia glumarum* to a possible alternate host. The discovery of such a host may also throw some

[1] Cooperative investigations between the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Approved for publication by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 63.

[2] The writers wish to express their appreciation to Dr. C. W. Hungerford, Plant Pathologist of the Idaho Agricultural Experiment Station, for valuable suggestions during the progress of these investigations, and to Dr. H. B. Humphrey, Division of Cereal Crops and Diseases, United States Department of Agriculture, for criticisms of the manuscript during its preparation.

much-needed light on the question of the present distribution of stripe rust in North America. This alternate host, if there be one, may be adapted only to climatic and soil conditions more or less peculiar to the Pacific and Intermountain States. These investigations included, in addition to the urediniospores of *P. glumarum,* those of *P. graminis* and *P. triticina.* The teliospore-germination tests were confined solely to teliospores of *P. glumarum.*

Stripe rust has long been recognized as one of the most destructive cereal diseases in Europe, its importance there being comparable to that of bunt of wheat in the United States. Reports from the major wheat areas of Argentina show the occurrence of stripe rust in moderate to severe epiphytotics (14). Although stripe rust is known to occur in the United States only in the Pacific and Intermountain States, it is potentially very important to the major wheat-growing areas east of the Rocky Mountains where, by chance, it may some day be introduced by interstate crop transportation or from other parts of the world where stripe-rust epiphytotics commonly occur.

The severity of stripe rust in the Western Hemisphere is dependent largely on climatic conditions of the current crop year. In 1928 H. B. Humphrey of the United States Department of Agriculture and the senior writer inspected wheat fields in the Flathead Valley of Montana where stripe rust was present in severe epiphytotic form. Other observations, made in various sections of the Pacific Northwest, indicated no little damage from this rust.

In the apparent absence of the aecial stage of *Puccinia glumarum* there is sufficient evidence to show how the organism might overwinter. Eriksson and Henning (6) were of the opinion that the severity and extent of an epiphytotic were more directly dependent on hibernating mycelium than on surviving urediniospores. Hungerford (15), Rostrup (22), and Hecke (9, 10) agree that the mycelium is able to withstand adverse conditions. Although the evidence presented by Biffen (2) and Klebahn (16) is not so convincing, they claim that urediniospores play a part in the perpetuation of the fungus and may subsequently act as a source of infection. Hungerford (15), however, reports the finding of viable urediniospores during the months of September, 1917, to July, 1918, in western Oregon.

GERMINATION OF UREDINIOSPORES

Historical: Information concerning the germinability and longevity of urediniospores of *Puccinia glumarum* is very meager. Early European data are limited and confusing. Always there is a question regarding the identity of the particular species of the rust under consideration. Eriksson and Henning (6), quoting the data of earlier investigators or that of

their contemporaries, state that the germination of urediniospores lasts from 2–3 days (Wolff, 1875) to 8 months and 12 days (Barclay, 1891). Eriksson (7), himself, secured good germination of urediniospores of *P. glumarum* after cooling them for some time.

Probably the most accurate data available are those recorded by Hungerford (15), who states that "germination tests with urediniospores of stripe rust show that when the leaves of the infected host are kept in herbarium packets at ordinary room temperature the spores may remain viable at least 58 days. Urediniospores on leaves of wheat kept in open vials in a desiccator gave a slight percentage of germination at the end of 63 days. Urediniospores taken from wheat leaves, placed on glass slides and kept in a protected place in the laboratory, gave a trace of germination in 23 days."

Spore-Germination Technique: Melhus and Durrell (18) obtained a higher percentage of germination of urediniospores of *Puccinia coronata* in distilled or redistilled water than they did in tap water. Tap water at Ames, Iowa, was distinctly toxic to the urediniospores of *P. coronata.*

In an effort to discover a satisfactory method of germinating the urediniospores of *Puccinia glumarum* for subsequent use in spore viability studies at Moscow, some experiments involving the employment of ordinary tap water, distilled water, and rain water were conducted. In the preliminary attempts the percentage of germination in tap water was higher than in distilled water. In these tests uredinial material was stored at different temperatures. Two spore cultures were made for each temperature; one in distilled water, the other in tap water. These cultures were made merely by scraping the spores from the leaves into a drop of water on a glass slide and covering them with a cover-glass. The slides were placed in Petri dishes lined with moistened filter-paper and then placed in the refrigerator. No attempt was made to culture spores of the same age. In view of the fact that absence of free air inhibited germination, the cover-

TABLE 1.—*Comparative effect of distilled and tap water on the germination of urediniospores of Puccinia glumarum at Moscow, Idaho*

Storage temperature ° C.	Number of trials	Percentage of germination in distilled water	Percentage of germination in tap water	Number of spores counted
3	2	10.2	14.7	2,000
12	2	18.0	24.6	2,000
15	2	14.2	19.4	2,000
22	2	10.8	15.3	2,000
30	2	6.0	8.7	2,000

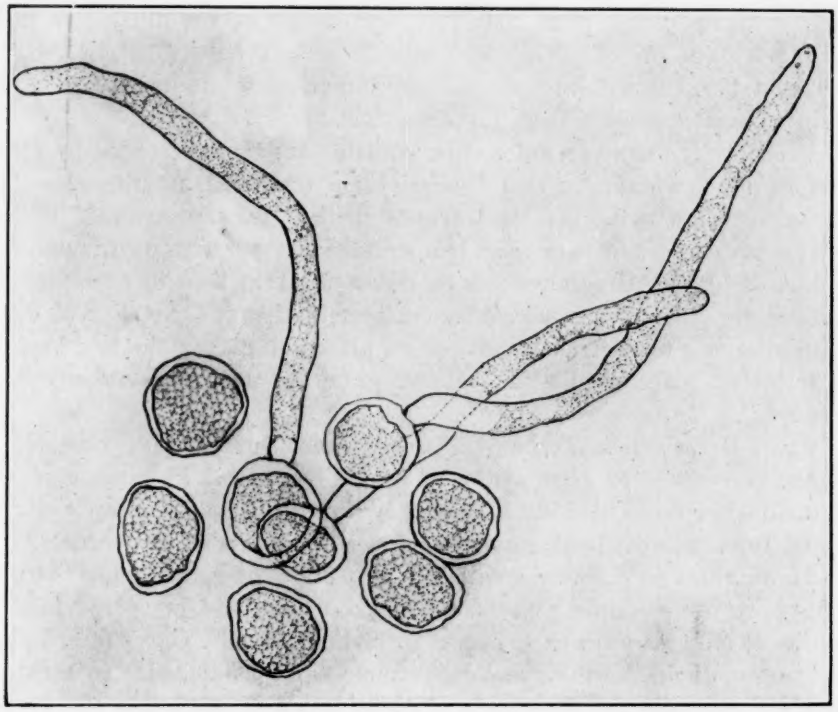

FIG. 1. A camera-lucida drawing of germinating urediniospores of *Puccinia glumarum*. These germinating spores are representative of those described as being kept in an air-tight chamber (Fig. 2) containing a relative humidity of 49 per cent and a temperature of 9° to 13° C. ×1,250.

glass was discarded in subsequent tests. Table 1 and figure 1 show the effect of the medium on germination and results obtained in the first attempts to germinate urediniospores.

Subsequent attempts to germinate urediniospores in rain water and in tap water gave 9.8 per cent germination in the one and 35 per cent in the other. A total of 4,000 spores was counted in determining these percentages.

Longevity of urediniospores: Peltier (20) states that the highest percentage of germination and the longest viability period of the urediniospores of form 3 of *Puccinia graminis tritici* occurred at a medium relative humidity. The results Hungerford (15) obtained with the urediniospores of *P. glumarum* have already been quoted. Hart (8) states that the optimum temperature for urediniospore germination of *Melampsora lini* (Pers.) Lev. is about 18° C. Fairly abundant germination obtains within the range of 6° to 23° C. The minimum lies near 0° C. and the maximum

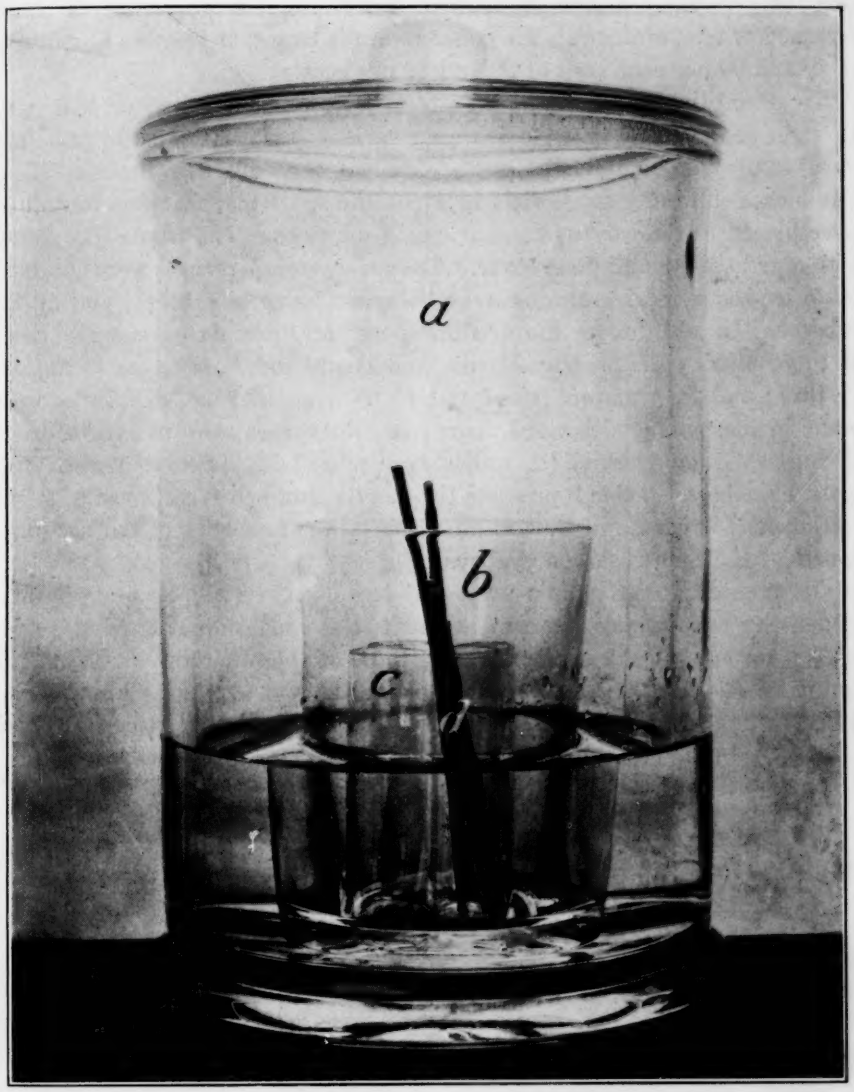

FIG. 2. *a*, A large glass chamber with a ground-glass top, containing a solution of sulphuric acid and distilled water of the desired concentration to create the necessary humidity; *b*, an ordinary glass tumbler in which are placed four glass vials; *c*, four glass vials, receptacles for the spore-bearing material; *d*, wheat-culm sections, placed in the vial to show arrangement of the spore-bearing material.

temperature is about 26° C. Under favorable conditions these spores retain their viability almost 3 months. They lose it more rapidly at high than at low temperatures. They also retain it longer at relative humidities of 40 and 60 per cent than at 20 and 80 per cent.

In attempting to determine the effect of various humidities on longevity air-tight chambers (Fig. 2) containing various strengths of sulphuric acid were employed. Humidities were obtained according to Stevens (24). Tumblers containing small vials in which the spore material was contained were placed in the various sulphuric-acid solutions. The whole was sealed with a greased ground-glass cover. The several temperatures were obtained in an ice room, refrigerator, storage basement, open laboratory, and an incubator. In addition to the urediniospores of *Puccinia glumarum,* those of *P. graminis phlei-pratensis* Eriks. and Henn. and *P. triticina* were used in 1927; and *P. graminis tritici* and *P. triticina* in 1928. Cultures were made in the manner described above; *i.e.,* the spores were placed in drops of tap water on glass slides which were placed in improvised Petri-dish moist chambers. Table 2 presents the results obtained from 2 years' study of the effect of temperature and humidity on the longevity of the urediniospores of the four rusts listed above.

In examining the results presented in table 2 it will be noticed that, under the conditions of the experiment, the urediniospores of *Puccinia glumarum* are not so long-lived as are those of the other three rusts. The spores of *P. glumarum* remained viable 88 days at a relative humidity of 49 per cent and a temperature of 9° to 13° C. Those of *P. graminis phlei-*

TABLE 2.—*The effect of various temperatures and relative humidities on the longevity of urediniospores of Puccinia glumarum, P. graminis phlei-pratensis, P. graminis tritici, and P. triticina, at Moscow, Idaho, in 1927 and 1928*

Relative humidity	OVEN							
	29° to 30° C. 1927 and 1928							
	Puccinia glumarum				*P. triticina*		*P. graminis phlei-pratensis*	*P. graminis tritici*
	Loose spores		Spores on leaf					
	Number of days urediniospores remained viable							
	1927	1928	1927	1928	1927	1928	1927	1928
100	Mold	Mold	Mold	Mold	Mold	Mold	Mold	Mold
76	6	21	0	8	30	32	9	23
49	6	28	11	35	32	35	26	32
25	0	14	5	25	20	25	26	28
1.5	0	25	0	23	4	18	21	18
Control[a]			14	16				

	DARK ROOM							
	13° to 26° C. 1927—23° to 26° C. 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	3	Mold	Mold	Mold	4	Mold	2	Mold
76	10	36	20	32	21	32	28	25
49	8	18	44	42	33	35	28	52
25	6	18	27	18	30	25	38	28
1.5	0	18	3	11	16	21	26	34
Control			31	25				

	REFRIGERATOR							
	9° to 13° C. 1927 and 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	14	11	4	11	8	4	7	5
76	16	53	51	69	75	104	111	124
49	12	60	75	*88*	107	118	*120*	*128*
25	10	53	35	63	77	110	117	114
1.5	0	52	30	56	36	71	117	62
Control			31	45				

	BASEMENT							
	9° to 21° C. 1927—3° to 11° C. 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	14	4	4	4	3	6	15	5
76	16	57	44	68	63	118	69	120
49	12	60	53	78	88	*124*	88	126
25	12	48	33	60	69	105	76	110
1.5	0	39	33	41	42	71	62	73
Control			16	22				

	ICE ROOM							
	−2° to 5° C. 1927 and 1928							
	1927	1928	1927	1928	1927	1928	1927	1928
100	5	4	2	8	11	8	7	5
76	12	23	14	48	21	49	9	35
49	10	27	15	48	17	44	26	42
25	6	12	6	13	19	32	16	35
1.5	0	0	4	12	11	8	26	8
Control			7	38				

[a] Material in packets, held at the various temperatures, outside the humidity chambers.

pratensis and *P. graminis tritici* under the same conditions remained viable 120 and 128 days, respectively. Urediniospores of *P. triticina* remained viable 124 days in a relative humidity of 49 per cent and at a temperature of 3° to 11° C.

It will be noted also that a considerable difference in results was obtained in the 2 years' tests. In nearly every case the period of viability of each rust was longer in 1928 than in 1927. Two factors may have been responsible for this. First, the age of the spores at the beginning of the tests may have influenced the results. Second, the temperatures were held more constant in 1928 than in 1927.

In 1928, when those spores, held at a relative humidity of 49 per cent and at a temperature of 29° to 30° C., had ceased germinating, they were subjected to temperatures of 9° to 10° C. without removing them from the original humidity chambers. Cultures were made after the spores had been exposed to the latter temperature for 48 hours. The urediniospores of all three rusts resumed germination. Those of *Puccinia glumarum* germinated throughout 6 more days; those of *P. triticina,* 8 more; and those of *P. graminis tritici,* 11 more days. The spores in the 49 per cent humidity chambers, held at near zero C., also were transferred to room temperature (23° to 26° C.), after the spores had ceased germination at the original temperatures. After 48 hours' exposure, germination was resumed. The spores of *P. glumarum* germinated for 4 more days; those of *P. triticina,* 6 days; and those of *P. graminis tritici,* 6 days.

GERMINATION OF TELIOSPORES

Historical: Eriksson and Henning (6) were undoubtedly the first to report the germination of the teliospores of *Puccinia glumarum.* Prior to their investigations, according to them, it was commonly believed that the teliospores of the collective species *P. rubigo-vera* germinated only after a resting period, *i.e.,* during the spring following the season of their formation. These investigators, having separated *P. rubigo-vera* into several species, discovered that the teliospores of *P. glumarum,* unlike those of *P. rubigo-vera,* germinated without having to pass through a dormant period. Not all of the spores germinated in the fall. Eriksson and Henning (6) observed that a small percentage of teliospores of *P. glumarum* would germinate immediately after they had formed, while others apparently laid dormant until the following spring or fall. This, however, is not an unusual phenomenon in the genus Puccinia, for the spores of *P. dispersa* Eriks. and Henn. (17) possess the same ability. Other cereal rusts, such as *P. graminis* (7), *P. coronata* Corda (11), and *P. triticina* (17), produce teliospores that require a resting period before they will germinate.

Very little has been done to determine the viability of teliospores of *Puccinia glumarum* as well as the optimum conditions for their germination, together with sporidial formation. In view of these facts, a rather comprehensive study of this phase of the problem was undertaken. Such a study was intended to give definite data on the optimum conditions for germination of teliospores and sporidial formation and to serve as a preliminary procedure necessary to any proper investigation of the relation of *P. glumarum* to possible alternate hosts.

Preliminary teliospore-germination tests: Teliospores of *Puccinia glumarum* were first germinated, after a number of preliminary trials, in the fall of 1926. Material was collected on September 5, 1926, and tested the same week merely by placing the spores in tap water on a glass slide in a Petri dish lined with moistened filter-paper. This improvised moist chamber was then placed in the refrigerator. The spores were taken from *Hordeum jubatum* L. The percentage of germination was not determined. This method differed somewhat from that of Eriksson and Henning (6), for they did not remove the spores from the host matrix but permitted them to remain intact. The spore-bearing material was cut into small pieces and placed in water or in contact with moist soil.

Because of the response obtained with the spores from *Hordeum jubatum*, as noted above, another preliminary test was made with teliospores from *Cinna latifolia* (Trev.) Griseb. This specimen was collected on September 12, 1926. The spores were tested the following day and germinated within 2 days. The percentage of germination was again undetermined. In neither of the above cases would the percentage of germination have exceeded 5 per cent.

In an endeavor to determine the course of the viability of teliospores the senior writer collected telial material of *Hordeum jubatum* in the grass garden at Moscow, Idaho, on September 21, 1926. As is generally the case, the spores on this host developed on the leaf sheath, and this facilitated the experimental use of the material. The latter was divided into two groups and stored as follows: Group 1—in refrigerator; group 2—in incubator (28° C.).

Periodic germination tests were made with this material beginning September 21, 1926. On September 27, 1926, cultures were made from the rust-infected *Hordeum jubatum* collected September 5, 1926, and that collected September 21, 1926. In both cases the percentage of spores germinating was equivalent to that in the original trials.

Table 3 gives in detail the dates on which cultures were started and results obtained with groups 1 and 2, noted above. The same spore-culture procedure as that described in the foregoing was followed throughout the

studies here reported. Germination of the spores apparently had ceased by February, 1927. There were, therefore, no further cultures made except after the material was treated as described below.

On October 4, at the time spore cultures of groups 1 and 2 were made, a culture of teliospores was made from infected Jones Fife wheat collected by W. L. Popham at Bozeman, Montana, on August 20. The specimen from which the culture was made had been kept in the laboratory from the time it was received. At the end of 24 hours germination resulted only with the spores from groups 1 and 2. Of these, the spores stored in the incubator showed the highest percentage of germination. At the end of 72 hours a few spores from the wheat plant had germinated and there was an increase in the number of spores germinating in the other two cultures.

TABLE 3.—*Germination of teliospores of Puccinia glumarum that had been stored at different temperatures at Moscow, Idaho, in 1926 and 1927*

Date of starting culture	Group No.	Germination results				
		24 hours	48 hours	72 hours	96 hours	120 hours
1926						
Oct. 4	1[a]	+	Increase	Increase		
	2[b]	+	"	"		
Oct. 25	1	0	+			
	2	0	+			
Nov. 2	1	0	+	Same		
	2	0	0	0		
Nov. 8	1	0	+	Same	Same	
	2	0	0	0	0	0
Nov. 15	1	0	+	Same	Same	
	2	0	Slight	Same	Same	
Nov. 22	1	0	0		0	0
	2	0	0		0	0
Nov. 29	1	0	Slight			
	2	0	"			
Dec. 7	1	0	+			
	2	0	0			
Dec. 15	1	0	+			
	2	0	0			
Dec. 30	1	0	0		Few	
	2	0	0		"	
1927						
Jan. 10	1	0	0			
	2	0	0			
Feb. 28	1	0	0		0	0
	2	0	0		0	0

[a] Kept in refrigerator.

[b] Kept in incubator (28° C.).

Cultures from groups 1 and 2 were made on October 25. Examination of the cultures at the end of 48 hours showed germination in both, a greater number of spores germinating in that culture taken from the material stored in the incubator.

Germination tests of groups 1 and 2 were again started on November 2, 1926. At the end of 48 hours no spores had germinated in the culture from the material stored in the incubator. Good germination was obtained in the culture from the material stored in the refrigerator; in fact, it compared favorably with that of the various cultures made when the experiment was started. At the end of 72 hours neither culture from the incubator-stored material showed any germination. Germination in the culture from the refrigerator-stored material remained the same as at the end of the 48-hour period.

The fact that there was no germination in group 2 at the end of the 72-hour period and that there was the usual amount in group 1 at the end of 48 hours, notwithstanding the fact that previously there was a higher percentage among the spores of group 2, leads one to believe that such differences may have been due to differences in spore age. The spores tested were never taken from the same two culms twice in succession. When enough were obtained and mounted on a slide, the culm from which the spores were taken was discarded and a new culm from each group was used each time a test was made. Accordingly, each time a test was started spores were used and compared that might have varied in maturity. On the other hand, this difference in response shown by spores stored under two differing sets of conditions might have been directly due to this difference in environment.

The walls of many of the spores seemed quite impervious to water and there was no consequent swelling of the spores. They appeared to be of a healthy yellow color and normal cell content. Often one cell of a teliospore would increase in size, indicating the absorption of water, while the other would still retain its normal size and appearance. All spores that germinated were larger than those that did not.

On November 8, 1926, the cultures of groups 1 and 2, begun on November 2, were exposed and allowed to dry 24 hours. At the end of this time water was again added to the slides and the moist chambers and they were again placed in the refrigerator. No spores had germinated at the end of 24 hours. New cultures of groups 1 and 2 were started on November 8. At the end of 48 hours germination was noticed only in the culture from group 1.

The two cultures of November 2, allowed to dry for 24 hours, beginning November 8, showed no germination at the end of 72 hours. They were again exposed and allowed to dry.

When cultures of November 8 were examined, 96 hours later, germination was observed only in the culture from group 1. In this culture the percentage of germination of spores suspended separately in the water was quite negligible. The greatest amount of germination was noticed in the spore aggregates still adhering to portions of the host. Groups of spores free from any host matrix showed little, if any, germination. At the end of 120 hours no differences were noticed in the culture of November 8.

The cultures of November 2, dried on November 8, moistened November 9, and dried November 12 were again moistened and placed in the refrigerator on November 13. Forty-eight hours later none had germinated.

On November 15, 1926, cultures were again made of groups 1 and 2, also of teliospores from infected barley C. I. 1315, collected in the greenhouse on November 13, 1926. Of the above, a few spores of group 2 had germinated at the end of 48 hours, more from group 1, and none from the barley. At the end of 72 hours some of the spores from the barley had germinated. No change was observed in the cultures from groups 1 and 2. At the end of 96 hours more spores from the barley had germinated.

Groups 1 and 2 were again cultured on November 22, 1926. No germination was observed at the end of 96 hours, so the cultures were discarded. The culture from barley started November 15 still showed viable spores. Many of the older germ tubes had lengthened and ramified. The contents of the germinating cell of the spore was generally to be found in the growing tip of the promycelium and was distinctly granular, an observation which conforms to the finding of Eriksson and Henning (6). Cultures of groups 1 and 2, made November 29, 1926, each, contained a few germinating spores at the end of 48 hours. A culture from the barley collected in the greenhouse November 13, made on November 29, failed to respond at the end of 48 hours.

It was noticeable that the number of teliospores responding had gradually decreased. None of the more recent cultures showed the amount of germination that had been obtained with the same material in September.

Cultures were again made on December 7, 1926, from groups 1 and 2 and from barley C. I. 1315, collected in the greenhouse on November 13. No spores had germinated in any of these cultures at the end of 48 hours.

Cultures of groups 1 and 2, started December 15, 1926, showed germination only in the case of group 1 at the end of 48 hours. A culture from *Hordeum jubatum,* collected December 3 and started December 15, showed no response at the end of 48 hours.

On December 18, 1926, teliospores were collected on *Hordeum jubatum,* in the grass garden at Moscow, and cultured the same day. At the end of 48 hours none of the spores had germinated.

Groups 1 and 2 were again cultured on December 30, 1926. A few spores of each group had germinated at the end of 96 hours.

To test the germinability of old teliospores that had been stored in the laboratory from date of collection, cultures were made of spores collected at Moscow on *Agropyron cristatum* (L.) Gaertn., in the summer of 1920, and of teliospores collected at Moscow on *Hordeum jubatum* July 1, 1925. No germination was noticed in either group of cultures at the end of 72 hours.

Groups 1 and 2 were again cultured on January 10, 1927. No spores had germinated in either culture at the end of 48 hours.

Teliospores collected on January 28, 1927, on barley C. I. 1315, grown in the greenhouse, were cultured January 31 in the usual manner. No spores had germinated at the end of 48 hours nor at the end of 72 hours. Within a week a few spores had germinated and further germinations occurred until the end of the eleventh day, when the culture was discarded.

On February 28, 1927, cultures of both groups 1 and 2 were made. At the end of 5 days no germination was observed in either culture. Most of the spores on the slide from group 1 appeared abnormal, in that they lacked the normal yellowish brown color, and many seemed devoid of the characteristic granular contents. The spores on the slide from group 2 were normal in every respect.

The reaction of those teliospores that germinated and failed to produce sporidia was in all probability due to faulty technique. A higher percentage of germination occurred near the edge of the cover-glass where air content of the water was greatest. Those spores that did germinate nearer the center of the cover-glass produced longer and more tortuous germ tubes. These results closely approximated those of Melhus, Durrell, and Kirby (19) who, in working with the teliospores of *Puccinia graminis,* state that a relative humidity of at least 95.6 per cent is required for teliospore germination and the production of sporidia. In a humidity of 100 per cent sporidia are more profuse. Teliospores submerged in a drop of water produce long attenuated germ tubes and sporidia production is slight or wanting. Subsequent tests indicated that the cover slip placed over each culture produced conditions that inhibited germination. Cultures in which the cover slip was absent gave a higher percentage of germination and a profuse production of sporidia.

Effect of various stimuli on the germination of teliospores: For some time previous to the date of the last attempts to germinate the spores, February 28, 1927, it was noticed that those spores that had been stored in the refrigerator remained viable longest. But, even in this group, the percentage of germination was very small. Because of this and the fact that the cultures showed so many water-impervious spores, it was proposed to

treat them in various ways in an attempt to penetrate the impervious cell walls, if such they be, and thus stimulate such spores to germinate or possibly revive this capacity in others.

Chemical stimuli: Acids: Thiel and Weiss (25), working with the teliospores of *Puccinia graminis tritici,* induced them to germinate prematurely by exposing them to a 1 per cent solution of citric acid for 15 minutes. Durrell (5) stimulated the spores of *Basisporium gallarum* Moll. to germinate by exposing water-drop cultures of spores to CO_2 in concentrations of from 1 to 5 per cent. Howe (12) secured the highest percentage of germination with the spores of *Ustilago levis* (K. and S.) Mag. in a medium having a pH of 4.9 secured by a continuous flow of CO_2 into the spore suspension.

In the light of these investigations it was decided to expose teliospores of *Puccinia glumarum* to a 1 per cent solution of citric acid. On March 11, 1927, teliospores of group 1 were treated with a 1 per cent solution of this acid for 15 and 30 minutes, respectively. This was accomplished by submerging in the acid spore-bearing portions of the host culm. After removing from the acid and rinsing thoroughly in tap water, the spores were cultured in the usual manner.

In less than 24 hours excellent germination was obtained with the spores which had been exposed to the acid for 15 minutes. A few spores had germinated in the other culture. At the end of 72 hours the number of spores germinating in the culture which had been exposed to the acid for 15 minutes gave the highest percentage of germination so far secured. Increased germination had resulted in the other culture but it was not so great as in the culture exposed to the acid for 15 minutes. This difference may be due not to the difference in treatment but to the fact that the spores used for the two cultures were obtained from different culms. Other conditions, therefore, such as difference in age of spore, thickness of spore wall, thickness of host epidermis covering the spores, etc., may have affected the germination of the spores.

In view of the reaction obtained with citric acid a number of other acids were used in 1 per cent concentrations. Only the spores from group 1 were used in these tests. The acids employed and results obtained with them are given in table 4.

It may be that the stimulating effect, if it could be called such, is due to the hydrogen-ion concentration rather than to the particular acid used. For example, the normalcy of the citric acid, which was a 1 per cent solution, was approximately .1/N, with an approximate pH value of 2.26. The hydrochloric acid was a 1 per cent solution of the commercial-concentrated product. It was about .1/N, with a pH value of 1.065. The use of the citric acid resulted in germination; that of the hy-

TABLE 4.—*The effect of various acids on the germination of teliospores of Puccinia glumarum on a single culm*

Treatment	Exposure	Germination results	
		24 hours	48 hours
Check. No treatment		0	3 spores
Boric acid, 1 per cent	15 min.	Good	Same
Hydrochloric, 1 per cent of commercial concentrated	"	0	Slight
Nitric, 1 per cent of commercial concentrated	"	0	Very good
Check. No treatment		0	0
Chromic, 1 per cent	"	0	Slight
Oxalic, 1 per cent	"	0	4 spores
Check. No treatment	"	0	Slight
Sulphuric, 1 per cent of commercial concentrated	"	Slight	"
Lactic, 1 per cent	"	"	"
Acetic, 1 per cent	"	"	Good

drochloric acid did not. The latter was, therefore, further diluted and spores were exposed to it for 15 minutes on March 18, 1927. Within 24 hours the spores thus exposed showed good germination, while only two germinating spores were noticed on the check slide. Within 48 hours the percentage of germination of the treated spores had materially increased. No increase in the number of germinating spores was noticed on the control slide.

On March 30, 1927, the same treatments were administered to the teliospores of group 2. The same procedure was followed in exposing the spores to the acids and the same culture technique was employed as in the preceding experiment. No germination resulted in any of the cultures.

On April 11, 1927, the spores of group 1 were again given the acid treatment for a period of 20 minutes instead of 15. The cultures were examined at the end of 24 and 48 hours and at the end of 1 week. In no case was there any germination.

The pH values of the various acids used ranged from 6.9 to 1.6, as determined with a La Motte No. 4 B Hydrogen-ion Testing Set.

Teliospores of *Puccinia glumarum* on *Aegilops cylindrica* Host. were first collected on July 15, 1927. Attempts to germinate them failed.

Fresh teliospore material was collected at Moscow on *Hordeum jubatum* on July 28, 1927. All attempts to germinate the spores resulted negatively. The effect of acids was again tested and, in addition to those named in the foregoing, the following organic acids were used: Tartaric, pH 2.1; maleic, pH 2.6; succinic, pH 3.3; salicylic, pH 3.7; phthalic, pH 2.7; and benzoic, pH 3.5. None of them induced germination.

Material collected July 28, 1927, was treated on August 23, 1927, with the various acids listed in table 3 for 15 minutes in the manner heretofore described; then they were cultured in the usual manner. One set of cultures was placed in the refrigerator, while a second set was allowed to remain in the laboratory. No germination was obtained in any of the trials.

Material stored in the ice box from date of its collection, August 2, 1927, was exposed to various dilutions of citric, oxalic, sulphuric, nitric, and hydrochloric acids on February 8, 1928. The spore-bearing culms of the host were immersed in the acids for intervals varying from 1 minute to 20 minutes, depending upon the concentration of the acid. The material was immersed in the weaker solution a longer period than in the more concentrated solutions. Nontreated controls were run for each acid. No germination was secured in any case. From the appearance of some of the spores after a week's incubation, partricularly those treated with the more concentrated solutions, it was apparent that the treatment was too severe.

Other chemicals: Rosa (21) obtained a mild stimulating effect on dormant potato tubers with ethylene in concentrations of from 1:400 to 1:2000 of air. He also found that solutions of 2 to 3 per cent sodium thiocyanate and ammonium thiocyanate were toxic and that ethyl bromide and ethylene dichloride hastened sprouting.

On November 20, 1927, material collected in July and held in the refrigerator was treated with ammonium thiocyanate pH 4.6, ethyl bromide pH 4.5, and ethylene dichloride pH 4.6, for 30, 60, and 120 minutes. Cultures from each treatment were made but gave only negative results. These treatments also were used on material that had been stored outside, but no germination was secured.

Teliospores, still adhering to the culms of the host, were placed in an air-tight chamber containing equal amounts of pure oxygen and ethylene gas. They were permitted to remain in this mixture of gases for 48 hours at room temperature. At the end of the 48-hour exposure the spores were cultured in the usual manner and incubated in the ice box for 72 hours. Six spores in the culture germinated, exhibiting a stronger and healthier germ tube than had been secured heretofore. A second trial gave negative results. The material used above was part of that collected July 28, 1927, and stored in the ice box from the date of collection.

As stated in the foregoing, it has been observed that when teliospores do germinate, they show a stronger and higher percentage of germination when attached to portions of the host or near the outer edges of the cover slip. It was assumed that this difference might be due to the more abundant oxygen in such localities. Durrell (5) noticed this same difference in working with *Basisporium gallarum*. Accordingly, hydrogen peroxide in 4 concentrations was employed in which teliospores were immersed for 15

minutes and then cultured in the same solution. Five, 10, 15, and 20 per cent solutions of the chemical were used. After incubating for 1 week in the ice box, readings were made. No effect was observed on the percentage of germination of the spores.

Exposing teliospores still adhering to the culms of the host to ether contained in desiccators had no stimulating effect upon germination. The spores were exposed to this gas for 12, 24, 48, and 72 hours, respectively, by placing them in the desiccators containing vials of sulphuric ether.

PHYSICAL STIMULI

Low temperatures: On October 14, 1927, telial material, collected the previous July and stored in the refrigerator since collection, was placed out of doors. The material was contained in a small gauze sack and hung from an east window. Up to January 16, 1928, cultures were made every 2 weeks. None, however, showed germination. At the time the material was placed outside the percentage of germination of the spores was 3. The temperature during this period ranged from 78° F. on October 20 to – 17° F. on December 17.

One might conclude from the above results that, unlike the teliospores of *Puccinia graminis,* which will germinate only when subjected to all changes of winter weather (4), those of *P. glumarum* are not in the least affected by such conditions. Later investigations, however, seem to contradict such a presumption.

Parallel with the biweekly tests made with the spores stored outside, the same type of cultures was made from the material still remaining in the refrigerator. These tests showed sporadic and unequal germination.

In the fall of 1928 fresh material was subjected to outdoor temperature in a manner similar to that employed in the winter months of 1927–28. From the results obtained there was an apparent stimulation. It will be noticed that with the tests of both years moisture was not considered. Being stored as it was, the material outside was never thoroughly wetted. It was held under air-dry conditions constantly.

Assuming that a combination of high humidity and freezing temperature might enhance germination, material was exposed to such conditions. It was first thoroughly wetted and placed between moistened filter-paper. Then it was exposed 5 days to a temperature ranging from – 2° C. to – 5° C. At the end of this period the spores were cultured in the usual manner.

Table 5 gives the results of these tests, including the name of the host, date and place of collection, and number of replications. It is quite evident that freezing alone does not induce teliospore germination. Moisture and low temperature combined seem necessary (Fig. 3).

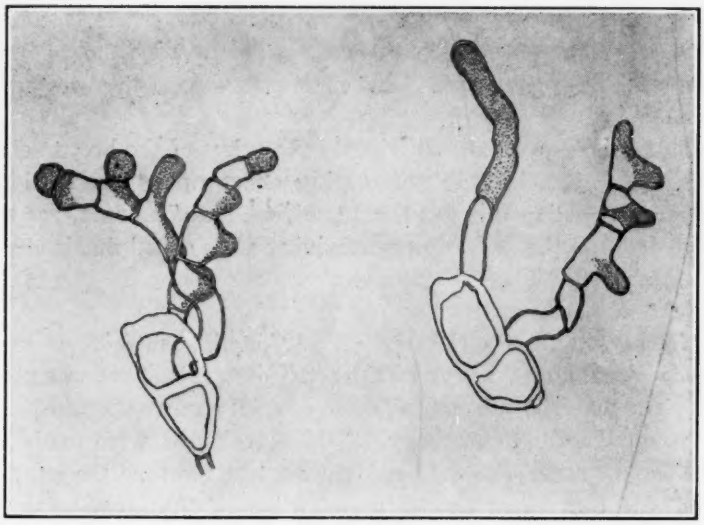

FIG. 3. A camera-lucida drawing of germinating teliospores of *Puccinia glumarum*. These teliospores were germinated in distilled water on open glass slides. The slides were placed in a moist chamber and allowed to remain in ice box for 48 hours. The drawings were made at the duration of that time. Drawings × 1,000.

Alternate wetting and drying: On November 17, 1927, spores still on the culms of *Hordeum jubatum* which had been stored in the refrigerator since July 28 and under outdoor conditions since October 14 were immersed in water for 24 hours. They were then removed and allowed to dry for 24 hours and again immersed for another 24 hours. When dry cultures were made and incubated in the refrigerator for 1 week, no germination resulted.

Alternating temperatures: Teliospores on culms of *Hordeum jubatum,* stored in the refrigerator, were transferred to an oven with the temperature at 30° C. They were left in the oven for 24 hours, then cultured in the usual manner and incubated 7 days in the refrigerator. About 3 per cent of the spores had germinated at the end of this time. Another lot of teliospores, placed in the oven at 30° C. for 48 hours, were then moved to the refrigerator for 24 hours. No germination resulted from this procedure. By another method cultures of spores were held in the refrigerator for 24 hours, then at room temperature for 48 hours. No germination was observed.

DISCUSSION AND CONCLUSIONS

From the results obtained by Becker (1), who found that urediniospores of *Puccinia glumarum* had the ability to survive 433 days at 0° C. in a relative humidity of 40 per cent, it might follow that she studied a physiologic

TABLE 5.—*Low temperature and high humidity effects on the germination of the teliospores of Puccinia glumarum at Moscow, Idaho, 1928–1929*

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Treatment	Host	Date of collection	Place collected	Dates tested	Per cent germ.	Ave. per cent germ.
Frozen 5 days	*Hordeum jubatum*	Sept. 20, 1926	Grass garden Exp. Station, Moscow, Idaho	10– 1–28	0	
				10–10–28	0	
				10–20–28	0	0
				11– 5–28	0	
				1–21–29	0	
Check	"	"	"		0	0
Frozen 5 days	Jenkins Club wheat	July 20, 1927	Agronomy plots, Expt. Sta., Moscow, Idaho	10– 1–28	3	
				10–10–28	4	
				10–20–28	3	3
				11– 5–28	2	
				1–21–29	Tr.	
Check	"	"	"		0	0
Frozen 5 days	"	July 25, 1928	Agronomy plots, Expt. Sta., Moscow, Idaho	10 –1–28	15	
				10–10–28	20	
				10–20–28	11	14
				11– 5–28	10	
Check	"	"	"		0	0
Frozen 5 days	*Agropyron tenerum longifolium*	June 16, 1928	Grass garden Expt. Station, Moscow, Idaho	10– 1–28	40	
				10–10–28	30	
				10–20–28	20	29.6
				11– 5–28	50	
				1–21–29	8	
Check	"	"	"		0	0
Frozen 5 days	Jones Fife wheat	July 8, 1928	Kalispell, Montana	10– 1–28	62	
				10–10–28	30	
				10–20–28	35	
				11– 5–28	38	35
				1–21–29	15	
				3–20–29	30	
Check	"	"	"		3	3

form of the rust that produced spores capable of withstanding conditions much more adverse than the spores of our physiologic form of the rust. In other words, Becker's results and those here presented afford further evidence that the form or forms in the United States are unlike those present in Europe.

The evidence on which to explain the absence of *Puccinia glumarum* in the United States east of the 103rd meridian is insufficient. Further studies of such phases of the problem as the effect of environment on spore germination, infection, incubation, and subsequent sporulation, and alternate-host studies of unconnected aecial rusts must be carried on before definite conclusions can be drawn.

The teliospores of *Puccinia glumarum* can germinate immediately on maturity without first having to pass through a resting period or be subjected to alternate freezing and thawing. It is not assumed, however, that such conditions are negative in their effect, for freezing teliospores of the stripe-rust fungus in the presence of moisture either revives this ability in spores which seem to have lost it, or stimulates it in others that apparently have not reached the proper state of maturity. All of the spores do not germinate during the season in which they were formed, for some still possess this ability during the spring following the previous fall's collection.

In respect to their ability to germinate during the season of their formation, the teliospores of *Puccinia glumarum* are not unlike those of *P. dispersa*. This characteristic seems unusual, for the teliospores of the more commonly known rusts, *P. graminis, P. coronata,* and *P. triticina,* do not possess this ability, but, on the other hand, require a period of dormancy before germination.

This early germinating ability exhibited by the teliospores of *Puccinia glumarum,* and the fact that the stripe-rust fungus can withstand adverse conditions by means of resting mycelium bring to mind the possible relationship that might exist in respect to the lack of a known aecial host. Ability to hibernate by means of dormant mycelium might preclude the necessity of an alternate host. Having no need for such a host the teliospores may have gradually lost their important function in the completion of the life cycle of the fungus. In doing so, they may have gradually acquired the ability to germinate immediately upon formation, rather than pass through a resting period, and the possible accompanying adverse condition, to await optimum conditions for germination and the appearance of a susceptible aecial host. Proof of the existence of an aecial host has yet to be established. If it does exist, it plays an unimportant rôle in the perpetuation of the fungus.

Of the various stimuli employed in trying to revive or stimulate germination of the teliospores, freezing temperatures, combined with high

humidity, resulted in the most pronounced stimulation. Herein are the teliospores of *Puccinia glumarum* similar in reaction to those of *P. graminis* and *P. coronata.* The stimulation resulting from acids or other chemicals in various dilutions was neither consistent nor conclusive.

Abundant sporidia develop if the right method of teliospore germination is used. This being the case, it is logical to assume that any possible lack of an alternate or aecial stage of the rust is not due to failure to produce sporidia.

SUMMARY

Stripe rust has long been recognized as one of the most important cereal diseases in Europe. In Argentina it occurs in moderate to severe epiphytotics. In the United States, so far as known, it occurs only in the Pacific and Intermountain States and is there only moderately severe.

Attempts to germinate urediniospores of *Puccinia glumarum* in tap water, distilled water, and rain water resulted in the highest percentage of germination taking place in tap water.

Under the conditions of the experiment the optimum humidity and temperature for the retention of germinability of the urediniospores of *Puccinia glumarum* were 49 per cent and 9° to 13° C., respectively. Spores held under these optimum conditions remained viable 88 days.

Urediniospores of *Puccinia graminis phlei-pratensis* and *P. graminis tritici* remained germinable under the same conditions for 120 and 128 days, respectively. Those of *P. triticina* were viable 124 days at a temperature of 3° to 11° C. and a relative humidity of 49 per cent.

Transferring the spores which had been held in the 49 per cent humidity containers at a temperature of 29° to 30° C., after all germination had ceased, to temperatures of 9° to 10° C., for 48 hours resulted in the renewed germination of the spores of all three rusts. The spores of *Puccinia glumarum* germinated 6 days longer; those of *P. graminis tritici,* 11 days longer, and those of *P. triticina,* an additional 8 days. In like manner, the urediniospores held at freezing and a relative humidity of 49 per cent were transferred, after they had ceased germinating, to room temperature (23° to 26° C.) and kept there for 48 hours. Under these conditions the urediniospores of *P. glumarum* germinated 4 days longer; those of *P. graminis tritici* and *P. triticina* 6 days longer.

Germination of teliospores of *Puccinia glumarum* was first obtained by the senior writer in September, 1926, from newly collected material.

At temperatures prevalent in an ordinary ice refrigerator, teliospores stored under such conditions retained their germinability longer that simultaneously collected spores held in an oven at 28° to 30° C.

Teliospores incubated under these conditions apparently had lost their ability to germinate by the following spring, but were revived by various stimuli.

Many teliospores present in the cultures seemed to be impervious to water.

Tests indicated that an undetermined factor caused nonuniformity of germination in the cultures made from time to time. Indications were that this factor was difference in age of spores.

Various stimuli produced inconsistent results in an attempt to revive this power of the spores to germinate.

Immersing the spore-bearing material in a 1 per cent solution of citric acid for 15 minutes sufficed to induce the latent spores to germinate after they apparently had lost this ability.

Boric, hydrochloric, nitric, chromic, oxalic, sulphuric, lactic, and acetic acids, in 1 per cent or weaker solutions, seemed to stimulate germination.

Induced germination of teliospores was not the result of any certain pH value of the acids.

Fresh teliospores collected in 1927 did not possess the same germinating ability as did those collected in 1926.

Results obtained with ammonium thiocyanate, ethyl bromide, ethylene dichloride, ethylene-gas-and-oxygen mixture, and hydrogen peroxide were not conclusive in their ability to induce germination of teliospores.

Alternate wetting and drying of teliospores at ordinary temperatures did not induce germination, nor did freezing under air-dry conditions.

Exposing teliospores to a combination of high humidity and freezing temperature resulted in a marked increase in germination.

Abundant sporidia developed when the teliospores were germinated in an exposed drop of water on a glass slide.

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TRANSPIRATIONAL HISTORY AS A KEY TO THE NATURE OF WILTING IN THE FUSARIUM WILT OF PEAS[1]

MAURICE B. LINFORD

Among the hypotheses set forth by diverse investigators to account for the wilting characteristic of various Fusarium wilts, that which regards wilting as a result of failure of the water supply, by whatever agency this may be brought about, has frequently been mentioned. The frequently observed phenomenon of temporary wilting of affected plants during periods favorable for rapid transpiration is more readily explained by this than by other theories. In the Fusarium wilt of the pea (*Pisum sativum* L.) caused by *F. orthoceras* App. and Wr. var. *pisi* Linford[2] this alternate wilting and recovery has been seen only in large plants. In young plants it probably does not occur. When wilting begins it proceeds directly to the collapse and death of the wilting leaflets. Such sudden wilting may occur in plants grown in cotton-plugged culture tubes in indirect sunlight where the transpirational demand is slight, and, both in such tubes and in open pot culture, it is sometimes accompanied by a water-soaked appearance of collapsing parts. This wilting, coming as a sharp reaction from a condition of extreme turgidity which precedes it,[3] suggests that the loss of turgor may be not a consequence of an inadequate supply of water but rather a result of death of the cells and loss of their powers of retaining water.

Daily determinations of the transpiration rate of diseased plants during wilting should indicate which of these interpretations is the correct one. Accordingly, in the course of other investigations of the pea-wilt disease the following experiment was conducted to trace comparatively the transpirational histories of healthy plants growing in clean soil and of plants growing in wilt-infested soil throughout the entire course of the disease. Since it became impossible to repeat the experiment the data are not considered final. This report sets forth indicative evidence relative to the nature of wilting and suggests a mode of attack which may be made to yield highly important results.

[1] This paper is an offshoot of investigations of pathogenesis and resistance in the Fusarium wilt of peas conducted under a fellowship appointment, from the National Research Council, in the Department of Botany, University of Wisconsin. The writer gratefully acknowledges the helpful advice of Professor B. M. Duggar and the assistance of Mr. Oliver S. Orton in carrying on this work.

[2] Linford, M. B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

[3] See footnote 2.

Peas of uniformly susceptible pure line of the Alcross variety were planted individually in cylindrical glass tumblers which measured about 6.5 cm. in diameter by 9.5 cm. in depth, each containing 250 gm. of soil previously adjusted to approximately 60 per cent of its water-holding capacity. Twenty were prepared with clean, uncooked soil, and 20 with wilt-infested soil that, 1 year before, had been sterilized and inoculated with a pure culture of the wilt fungus.

As soon as the young sprouts had emerged, 12 of the most uniform of each lot were selected for the experiment. The seedlings were removed from 10 tumblers used as checks on water loss. A layer of melted wax[4] was then poured over the soil in each tumbler, reducing the possible channels of escape of water to the plant and the narrow glass-funnel tube which extended to the bottom of each tumbler for the addition of water during the experiment. In the check tumblers, in which these funnels and imperfections in the wax seals were the only channels of egress, weighings indicated that the water loss during the experiment was negligible.

Both lots of soil were adjusted to 60 per cent of their water-holding capacities before planting, and, then, before pouring the wax seals, each tumbler was restored to its initial weight by the addition of water. For convenience in weighing throughout the experiment, all tumblers were then adjusted to equal weight within the limits of 1 gram by the addition of warm lead shot (BB) which melted itself into the surface of the wax. The tumblers were then spaced equally on a wire-mesh support, 15 inches above the table top, in a large glass room where exposure to light, heat, and circulation of air was uniform. The temperature fluctuated chiefly between 20° and 24° C., reaching the limits of 15° and 27° C. during the experiment. Daily records of water loss, of the development of plants, and of wilt symptoms were made individually for each plant. Water was added from a burette while the tumbler stood upon one pan of a long-arm balance, counterbalanced by the initial adjusted weight of the tumbler. Thus the daily water loss per plant was read directly from the burette to tenths of 1 cubic centimeter, as water was added to restore that lost daily. On the twelth day after the wax seal was poured, total surfaces were estimated for every plant separately based upon a series of measurements of every leaflet, stipule, and stem.

The 12 plants in clean soil made vigorous and uniform growth as indicated by the uniformity of total surface on the twelfth day, recorded in table 1. Two plants in infested soil were attacked by Rhizoctonia and

[4] The most satisfactory wax seal was the following, modified from the formula of Briggs and Shantz: paraffin (Parowax), 5 parts; white petrolatum, 2 parts; and beeswax, 2 parts. When cooled to near its melting point before being poured, this wax caused no visible injury to the delicate sprouts.

dwarfed so badly that they were discarded. The remaining 10 showed fair uniformity in their development and also in the development of disease. Positive symptoms were noted first on the fifth day in 2 plants, and, by the ninth day, all 10 were diseased. By the eighteenth day, at the close of the experiment, the leaves and stem tips of all 10 plants in infested soil were fully wilted.

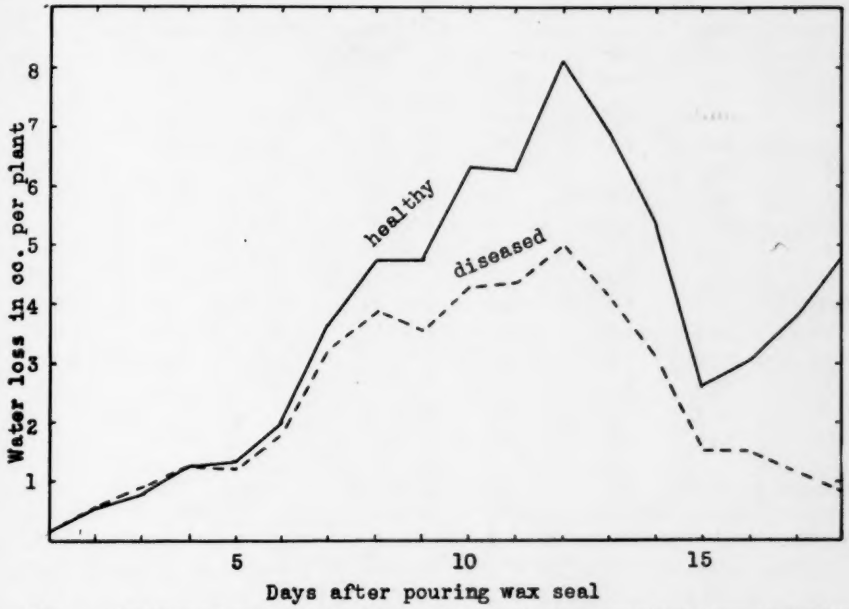

FIG. 1. Daily average loss of water, in cubic centimeters per plant, from 12 healthy and 10 diseased Alcross variety pea plants. First symptoms of the wilt disease appeared on the fifth day; and all leaves of all affected plants were fully collapsed on the eighteenth day.

With the progressive increase in transpiring surface of the healthy plants, the daily loss of water should have increased progressively, but, as shown in figure 1, this was not the case. A sharp reduction occurred in the transpiration of both healthy and diseased plants between the twelfth and the fifteenth days as the result of a cold, cloudy period. This figure shows, however, that the daily loss from plants in the infested soil was slightly higher than from those in clean soil during the opening days of the experiment but that it gradually fell off during the succeeding days as the disease developed. This decline was the result of at least two factors as illustrated by table 1. In the infested soil the plants were distinctly dwarfed. On the twelfth day, when the collapse of affected plants had scarcely begun, the average surface exposed by the diseased plants was only 75.4 sq. cm. as compared with 96.4 sq. cm. exposed by the healthy

TABLE 1.—*Total areas and rates of transpiration of healthy pea plants and of plants in medium stages of Fusarium wilt. Areas were computed from measurements of every leaflet, stipule, and stem. These data are for plants of a susceptible pure line of the Alcross variety, on the twelfth day after the beginning of the experiment*

Healthy plants in clean soil				Diseased plants in infested soil			
Plant number	Total area	Water loss per day		Plant number	Total area	Water loss per day	
		Per plant	Per square meter			Per plant	Per square meter
	sq. cm.	*cc.*	*cc.*		*sq. cm.*	*cc.*	*cc.*
C–1	98.0	9.1	929	W–1	93.9	7.0	746
C–2	84.7	7.4	874	W–2	52.9	3.1	586
C–4	95.6	7.7	806	W–8	78.0	5.8	744
C–5	95.1	9.3	978	W–9	72.7	4.7	647
C–8	95.5	7.6	796	W–10	88.9	6.3	709
C–9	90.3	8.4	931	W–12	83.0	5.8	699
C–11	87.1	7.7	884	W–14	86.5	5.6	648
C–13	116.4	8.1	696	W–17	67.8	4.5	664
C–14	102.8	7.4	720	W–20	63.1	3.8	602
C–15	99.4	8.0	805	W–21	67.3	3.7	550
C–19	99.1	8.5	858				
C–21	92.3	7.7	834				
Average	96.4	8.1	838	Average	75.4	5.0	667

plants. But this smaller surface was not the only cause of reduced water loss, for, while the diseased plants showed 78.2 per cent as much surface area as the healthy plants, they lost only 61.7 per cent as much water. Computed to the basis of water loss per square meter of surface per day, as shown in table 1, the water loss from the healthy plants was 838 cc. as compared with 667 cc. from the diseased plants. These figures thus indicate that prior to wilting the diseased plants had a lower rate of transpiration than the healthy.

The daily trend of transpiration with progress of the disease is obscured in these figures by the wide fluctuation produced by environmental conditions. The daily average loss of water from the healthy plants was therefore adopted as a standard of comparison, and a transpirational ratio, $\frac{\text{water lost by diseased plants}}{\text{average water lost by healthy plants}}$, was computed. This ratio is strictly relative and is independent of daily fluctuations in the environment in so far as diseased and healthy plants respond similarly to environmental conditions.

Figure 2 represents graphically the daily transpirational ratios of 4 individual affected plants and the average of all 10 affected plants. In agreement with figure 1, it shows a general decline after the opening days

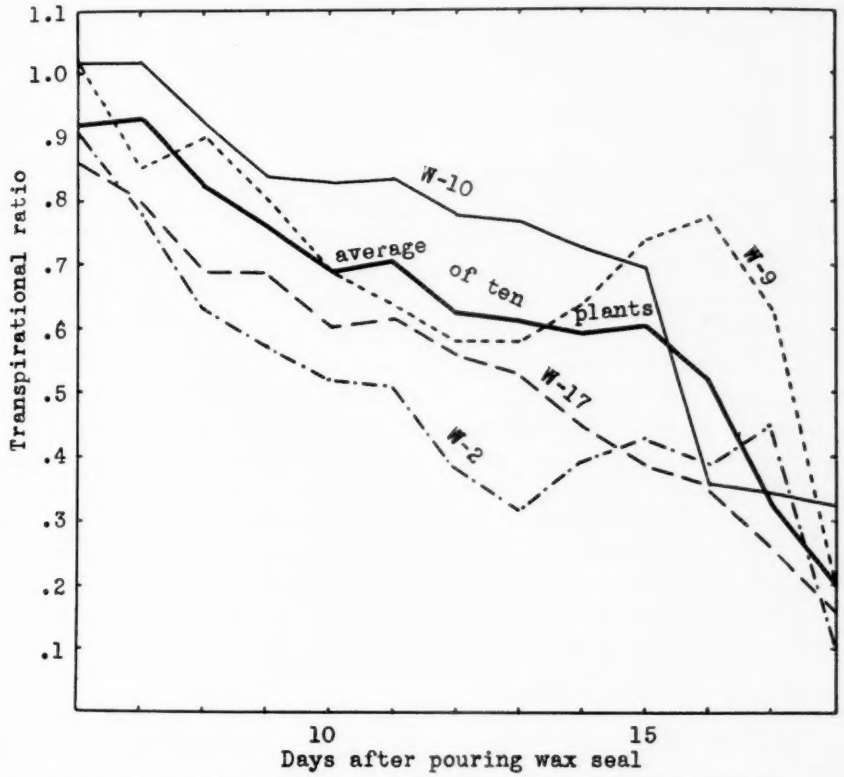

FIG. 2. Transpirational histories of pea plants affected with Fusarium wilt. Daily loss of water from 4 separate plants and average daily loss from 10 plants, expressed as the transpirational ratio; *i.e.*, $\frac{\text{water lost by diseased plants}}{\text{average water lost by healthy plants}}$. The 4 individual plant curves were selected to illustrate the limits of the observed variation between plants. Irregularities in the opening days of the experiment resulted from the large error involved in measuring small amounts of water transpired by the very young seedlings.

of the experiment, a decline which resulted both from the actual diminution in the rate of transpiration of diseased plants and from the increasing transpiring surface of the healthy plants. Our special interest lies less in this general decline than in any changes in rate of decline which may be coincident with late stages of the wilt disease.

Wilting was in progress in different individual plants from the thirteenth to the seventeenth day, and it is during just this period that the graph of the daily average transpirational ratio departs most distinctly from the otherwise nearly straight line. During this period, on the average, the diseased plants lost water more rapidly than was expected, only to show promptly a reduced rate of water loss after wilting was complete. This is shown more strikingly by the graphs drawn for plants W-9 and W-2. Both

these plants, during the several days of their wilting, showed a distinct increase in transpirational ratio, followed by a sharp decline after the leaves were all wilted. Clearly, in these two plants, wilting appears to have resulted not from a failure of the water supply but rather from a failure of water retention.

But not all 10 plants showed such a reaction. Plants W-10 and W-17, graphed in this same figure, are the exceptions. W-10 wilted rapidly on the fourteenth and fifteenth days, associated with a distinct reduction in the transpirational ratio such as would be expected if the wilting had resulted from water deficiency; and in W-17 the decline was gradual, graphing as a nearly straight line. Of the 10 diseased plants, 5 showed a distinct upward trend of the transpirational ratio coincident with wilting; 3 showed a questionable upward trend; and the 2 discussed here showed no such trend. The upward trend of the average curve is less decisive than inspection of the individual data suggests, due in part to failure of synchronization of the wilting in different plants.

In spite of their irregularity, however, these data seem to establish the fact that some plants, and probably most of them, suffer a rapid loss of water during wilting and that reduced availability of water, therefore, is not the chief factor, if even an important one, in the final wilting that characterizes the severe early development of this disease. Of more importance is some fundamental alteration of the protoplasts of leaf cells, probably their actual death, which leads to the loss of their normal powers of retaining water and consequently to their loss of turgor and to wilting of the leaf. That insufficient water supply may sometimes be involved is not disputed, particularly in the wilting of older plants. The rapid loss of water from collapsing leaves, indicated by this experiment, would subject the water supply of the plant to an extra strain and might lead, in plants with a reduced water-supplying capacity, to the wilting of other leaves from insufficient water. Such joint operation of these opposing factors might account for the behavior of plant W-17 indicated in figure 2.

SUMMARY

By following the transpirational histories of diseased and healthy plants the writer has obtained preliminary data which indicate that in the Fusarium wilt of peas wilting may result not from a diminished water supply but rather from an excessive loss of water from the leaves. This is thought to indicate the loss of the normal powers of water retention by the leaf protoplasts. It is sugested that this method of approach may yield significant facts regarding other vascular wilt diseases.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

STUDIES OF PATHOGENESIS AND RESISTANCE IN PEA WILT CAUSED BY FUSARIUM ORTHOCERAS VAR. PISI[1]

MAURICE B. LINFORD

INTRODUCTION

Although the wilt of peas (*Pisum sativum* L.) caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford has been recognized only a few years, it is now known to be one of the major diseases of canning-crop peas in the United States.[2] The writer showed earlier (7) that this is characteristically a vascular disease, comparable in many respects with the well-known vascular fusarioses of cabbage, flax, tomato, and certain other plants, and that, in common with these diseases, it presents a number of interesting and fundamentally important biological problems arising from the specialized form of parasitism represented. In continuation of this earlier work the writer has now made both histological and experimental studies bearing upon the questions of pathogenesis and resistance. The histological findings are being published in a later paper which traces the interrelation of parasite and host during the infection of susceptible pea plants and which considers the histological manifestations of disease and of resistance. An earlier paper (8) has already dealt with rates of water loss during wilting. This present paper deals with experimental and observational studies bearing upon the questions of infection and factors which control it, the nature of changes in the host plant which constitute the disease, means whereby these changes are induced by the pathogene, and possible factors in resistance. The present status of knowledge concerning the diseases other than pea wilt which are dealt with here has been summarized so adequately in recent publications (3, 9, 11, 14) that the writer has attempted no general review of literature.

One monoconidial culture (No. 182c) of the pea-wilt fungus, isolated by the writer from a wilting pea plant at Madison, Wisconsin, in 1926, was used throughout all but the initial stages of this work. The Badger variety of pea was used chiefly as the susceptible host plant and Horal as the resistant pea, with others introduced for comparison when needed. The seed came chiefly from the upper Snake River Valley of Idaho.

[1] This paper is the second of a series reporting studies conducted in the Department of Botany, University of Wisconsin, under a fellowship grant from the National Research Council. The writer wishes to express his appreciation to Professor B. M. Duggar for his helpful advice throughout the progress of the work.

[2] Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Plant Indus. Plant Dis. Rptr. Suppl. 67. 1929.

PATHOGENICITY OF THE WILT FUNGUS

Although there was no reason to doubt the causal connection between the fungus and the disease under consideration, still certain unexplained variations in the natural and experimental development of pea wilt, which the writer had observed, suggested that some undetermined complicating factors might be involved. As a safeguard against misinterpretation of histological and other studies the following experiments were conducted to ascertain more definitely the pathogenic capacity of the pea-wilt Fusarium. Since it had already been shown that soil temperature is a major controlling factor in pathogenesis (7) these studies were conducted within the range of temperatures favoring the rapid development of disease.

PATHOGENESIS IN PURE AND MIXED CULTURE

To test the possibility that an association of other microorganisms with the wilt fungus might be essential for the development of this disease, inoculations were made under pure-culture conditions in large cotton-plugged culture tubes (3.5 x 20 cm. and 5 x 40 cm.). Water agar, soil-extract agar, and soil were used as substrata. Selected seeds from a clean lot of Badger variety were surface-sterilized[3] and, as a test of sterility, were planted an inch apart in Petri dishes of soft potato-dextrose agar. After germination had begun, the most uniform and vigorous seedlings were transferred individually with flamed forceps to separate culture tubes. These large tubes of soft agar (prepared with either tap water or soil extract with 1 gram of dextrose added per liter) were inoculated with the wilt fungus at the time the germinating seeds were planted. Roots and mycelium then penetrated the soft agar together to a depth of several centimeters and infection of the roots resulted. When sufficient substratum was provided and when the plants were allowed to stand long enough, leaf symptoms developed that, while different in detail from those which characterize the disease in open pot culture, were sufficiently similar to indicate their essential identity.

In early experiments, inoculation of tubes of sterile soil at the time germinating seeds were introduced led to the complete rotting of a high percentage of the seedlings. This difficulty was overcome by introducing the wilt fungus 1 week before the seedlings. Mycelium developed rapidly during this time and became clearly visible in the soil. Thereafter, mycelial growth was less conspicuous.

To compare the development of wilt in pure and mixed cultures, large

[3] The following procedure proved satisfactory: Wet the seed in 70 per cent alcohol, soak 1 minute in mercuric chloride (1–1000), rinse and soak in sterile water for 2 hours, treat a second time in mercuric chloride for 30 seconds, rinse several times in sterile water, and drain.

culture tubes of soil were prepared as indicated in table 1. Sterilization consisted of autoclaving the cotton-plugged tubes at 15-pounds pressure on 3 successive days. Soil with uniform moisture content was used, and approximately this content was maintained by the addition of sterile water from a volumetric pipette to restore loss as indicated by weight. Germinating peas were introduced 1 week after inoculation of the soil, and then 3 days later these tubes were stood in a flat of moist sand in the greenhouse in indirect sunlight where the air temperature varied between 18° and 26° C.

From table 1 it is seen that the wilt disease developed only in series E and F, inoculated with the wilt fungus after sterilization of the soil, with or without the supplementary addition of raw noninfested soil. Even the addition of 5 cc. of infested soil to the sterile soil in series D was without apparent effect, for the two plants in this series were still healthy after 40 days. In series E, inoculated with a pure culture of *Fusarium orthoceras* var. *pisi*, the disease developed rapidly and more typically than in the agar cultures. All 6 affected plants developed symptoms that were clearly characteristic of the disease, although not identical with those observed in open culture. Root injury was more pronounced than is typical of the disease and involved extensive cortical decay. Vascular invasion was relatively limited and, where it did occur, was less restricted to the xylem and more general in the cambium and phloem than is typical of the disease. These departures from the usual development are regarded as due solely to the peculiar environmental conditions within the cotton-plugged tubes, which modified both structure and function of the host plant.

From these results it is clear that the wilt fungus is capable of independent parasitism. Although it is not established that other organisms are never involved in a mutual relationship, the failure to detect any beneficial influence upon the disease of such mixed populations as were obtained in the experiment probably means that such a possible relationship is at least rare and unimportant.

ROOT WOUNDS IN RELATION TO INFECTION

The weak cortical parasitism of the pea-wilt fungus suggested that wounds might aid its entry into the vascular system. This was tested in two types of experiments. In several different plantings, young seedlings of susceptible and resistant varieties grown in flats of infested soil were subjected to root pruning. A long-blade knife thrust vertically into the soil was used to remove the young lateral roots from one or more sides of the taproot. In no case did such treatment lead to an earlier development of symptoms in susceptible plants or destroy the resistance of resistant plants.

TABLE 1.—*Development of Fusarium-wilt symptoms in Badger peas grown 25 days from partially germinated seeds in large culture tubes of soil prepared as indicated; a comparison of pure and mixed inoculation*

Series	Number of plants	Soil and its preparation	Condition of experimental plants 25 days after planting the partially germinated seeds
A	4	Thoroughly infested from former experiments.	Seedlings all decayed before germination was complete.
B	2	Sterilized.	Both healthy.
C	2	Sterilized, with 5 cc. raw soil added.	One healthy with roots browned slightly; one dwarfed and pale with roots partly decayed.
D	2	Sterilized, with 5 cc. infested soil added.	Both healthy.
E	8	Sterilized, with culture of wilt fungus added.	Wilt symptoms in six plants: growth of apical buds checked, stems swollen and succulent, leaves of two plants wilting and those of four pale and distorted. Two plants apparently sound.
F	4	Sterilized, with raw soil and wilt fungus added.	Wilt symptoms of unilateral dwarfing with leaflet distortion in one plant; one plant dying from decay of hypocotyl; two died early from seedling rot.
G	2	Sterilized, with cultures of wilt fungus and *Pythium* sp. added.	Both healthy. (Both died later from Pythium rot.)

In the second type of experiment, plants were grown 16 days, to the 5- or 6-node stage, in clean soil and then transplanted to clean and to infested soil. These seedlings were removed from the soil with care to minimize root breakage, and the controls were replanted directly. The plants to be wounded, however, were pruned severely: the taproot was cut back to 50 mm., lateral roots were cut to 10 mm., and, to provide a corresponding reduction of leaf surface, petioles were cut away, leaving only the stipules. Five plants each of Badger and of Horal peas were planted pruned and unpruned in clean and in infested soil, making 40 plants in all. In the clean soil plants of both varieties, whether pruned or not, recovered rapidly. In the unpruned controls even the tips of most lateral roots were unharmed, while in the pruned plants the roots branched out freely and new leaves were formed.

In the infested soil all plants of the susceptible Badger variety developed characteristic symptoms of the disease; and this development was distinctly earlier in the unpruned controls than in the pruned plants. Dead

and dying roots were more numerous on the plants that had not been pruned. The resistant variety, Horal, developed no wilt symptoms in aerial parts following either treatment, although some dead rootlets were found on both pruned and unpruned plants. Microscopic examination of many wounded roots failed to disclose any significant entry of the fungus into the vascular system through a wound. Wounding of fairly mature roots as carried out in these experiments thus appears to be unfavorable to the early development of this disease and to have no effect upon inherent resistance. This latter point is in agreement with the recently published work of Wade (13).

INFLUENCE OF AMOUNT OF INOCULUM IN THE SOIL

When peas are planted soon after an abundance of pure culture of the wilt fungus has been introduced into sterilized soil, even at a temperature favorable for the disease, pea wilt often develops slowly and irregularly. A delayed planting or a second planting after the first peas have been removed from the soil usually leads to an early development of the disease. A somewhat comparable phenomenon has been observed under field conditions in the natural development of pea wilt. The disease tends to develop earliest in parts of a field in which it has been present former years. Thus the areas that are infested one year will commonly show an earlier death of plants in the next planting than will the surrounding areas into which the wilt fungus has spread during the intervening time. Two of the possible interpretations of these phenomena are (1) a direct relationship between abundance of the fungus and promptness of infection and (2) a gradual and deleterious modification of the soil as a result of the presence of the fungus. To test the former of these possibilities the following experiment was conducted.

Peas were grown comparatively in thoroughly infested soil and in such soil diluted to various degrees. The infested soil had been inoculated after sterilization 10 months earlier and had since been proved highly infective. Dilution at the time of planting was in two parallel series, (A) with fresh uncooked soil and (B) with similar soil sterilized in the autoclave 6 days earlier. The infested soil was diluted to the fractional concentrations indicated in table 2, and each diluted sample was sifted repeatedly through a $\frac{1}{8}$-inch-mesh sieve to insure thorough mixing. Badger peas planted in 6-inch clay pots of this soil were thinned upon emergence to 10 seedlings, some of which were then lost from damping-off.

Table 2 presents the results of this experiment and figure 1 illustrates representative pots after 25 days. Control plants remained healthy throughout the experiment; all plants in the full-strength-infested soil were fully wilted in 25 days. In both dilution series the disease developed

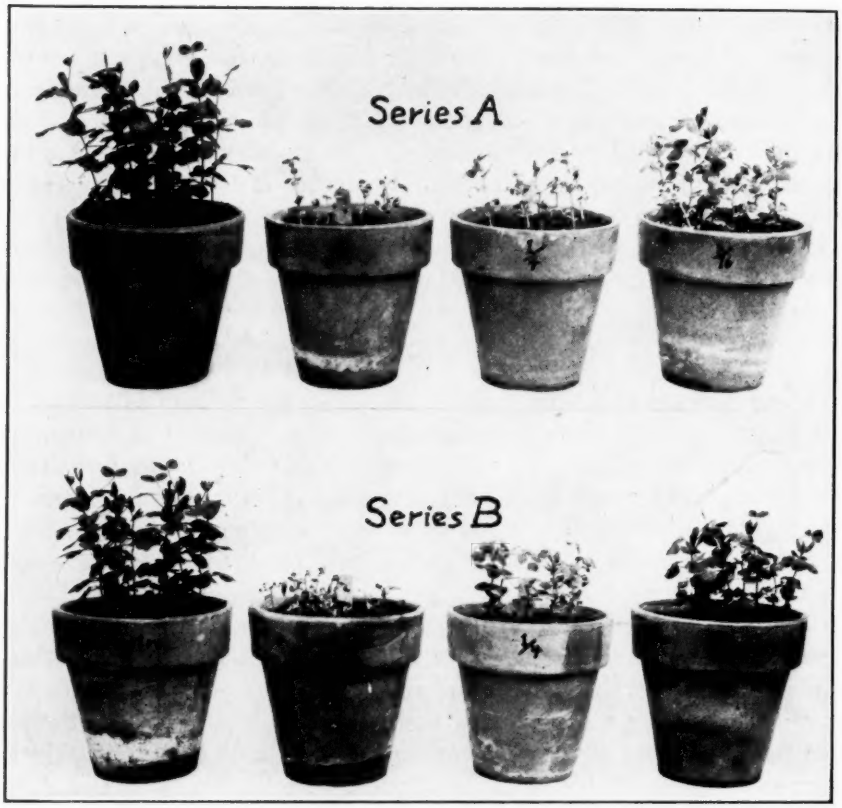

FIG. 1. Influence of concentration of inoculum upon the development of pea wilt. These photographs, taken 23 days after planting the seed, show progressive retardation of the disease with progressive dilution of the infested soil used as inoculum. Note that the control plants are larger in series A, diluted with uncooked field soil, and that the disease is more advanced in the various dilutions of this series than in series B, which was diluted with autoclaved soil. Compare with table 2.

more slowly as the degree of dilution increased; this was particularly conspicuous in series B, diluted with sterilized soil. Thus it is apparent that in plants exposed simultaneously to infection the disease may develop at very different rates, depending on the abundance of the wilt fungus in the soil or, at least, upon the proportion of infested soil in the soil mixture. This suggests that the rapid development of the disease in thoroughly infested soil may be a result of infection by the wilt fungus at many different points on the root system. The pronounced dwarfing which was observed in the greater dilutions (Fig. 1, B 1/16), even in the absence of other symptoms of the disease, suggests that in some way the wilt fungus may injure

TABLE 2.—*Influence of concentration of inoculum upon the development of Fusarium wilt of peas. Badger variety peas grown in the greenhouse where the temperature varied between 19° and 25° C.*

	Concentration of inoculum in soil at time of planting	Total plants	Days until first symptoms	Total plants affected after number of days indicated (per cent)					Plants wilted after number of days indicated (per cent)			
				17	19	22	25	27	19	22	25	27
Series A	Control	20	.....	0	0	0	0	0	0	0	0	0
Diluted with *fresh* field soil	1/1	6	15	100	100	100	100	100	0	100	100	100
	1/2	9	15	78	78	89	100	100	11	67	89	100
	1/4	7	15	57	86	86	86	86	0	14	71	71
	1/8	10	13	30	50	80	90	90	10	40	80	90
	1/16	9	15	33	56	67	89	89	11	22	44	67
	1/32	10	17	50	50	70	90	90	0	10	40	60
Series B	Control	19	.....	0	0	0	0	0	0	0	0	0
Diluted with *autoclaved* field soil	1/1	9	15	89	100	100	100	100	33	89	100	100
	1/2	5	15	40	60	100	100	100	0	0	60	80
	1/4	7	15	29	43	71	71	71	14	14	43	43
	1/8	2	27	0	0	0	0	50	0	0	0	0
	1/16	8	22	0	0	25	25	50	0	0	0	0
	1/32	5	22	0	0	20	20	40	0	0	0	0

the pea plant without entering the vascular system and thus completing infection.

MODIFICATION OF THE SOIL BY THE WILT FUNGUS

Some direct modification of the soil by the development of the wilt fungus might, if demonstrable, account for some of the variations indicated. Rosen (11) has suggested that the cotton-wilt fungus, *Fusarium vasinfectum,* which he reports has the property of reducing nitrates to nitrites, may cause nitrite injury to cotton roots and thereby produce the wilt disease without infection. His evidence seems uncertain at several points and Young, Ware, and Janssen (15) in later work found no support for the view. To test this with respect to pea wilt, several attempts were made to detect any possible toxic action of thoroughly infested soil which might aid in the development of early stages of the disease. Since only negative results were obtained, the work will not be described in detail. In one experiment, all water which reached the roots of plants grown in small porous cups passed in through the porous walls from an outer container of infested soil. In another, peas were grown in water culture in soil extracts from clean and from infested soil. In still another, infested soil was partially sterilized by various heat and formaldehyde treatments, but there was no development of symptoms in the absence of the fungus. Although these negative results are not wholly conclusive, they indicate very strongly that any influence upon the plant which the wilt fungus may have before it has become established in the vascular system of the roots is to be attributed to a direct action of the fungus developing upon the root surface or within the superficial layers of the cortex rather than to any indirect action through modification of the soil.

DISCUSSION OF PATHOGENICITY

These studies, although somewhat inconclusive at several points, suggest that the pathogenicity of the pea-wilt fungus is readily disturbed by various conditions in addition to the important controlling factor of soil temperature. The need for a well-standardized and ample inoculum in future studies of this disease is apparent, particularly in work that demands the use of sterilized soil, for it is clear that the rate of development of the disease may vary widely as a result of varied inoculation and that soil which has been sterilized with steam is relatively unfavorable for the disease. Such soil is likewise unfavorable for the healthy pea plant, which suggests again (7) that the disease develops best under conditions which, except for the presence of the wilt fungus, are most favorable for the growth of the healthy pea plant. The demonstration that the pea-wilt fungus is able to enter the vascular system and produce the disease without

the aid of other microorganisms and that such entry is not facilitated by gross wounds in the roots is in agreement with the earlier experience (7, pp. 34–36) that simultaneous infection with *Aphanomyces euteiches* Drechsler and the wilt fungus in no important manner affected the course of the wilt disease or lowered the degree of varietal resistance. These evidences, together with the narrow host range of the pea-wilt fungus, indicate that we are here dealing with a highly specialized type of parasitism.

It should be pointed out that recent advances in soil science, as recorded by Hendrickson and Veihmeyer (4, pp. 3–7), have shown the writer's earlier experimental work (7, pp. 14–17) on the influence of soil moisture on this disease to rest upon an unsound basis. The same is true generally of a great deal of phytopathological work in which essentially the same technique has been employed and the assumption made that different soil-moisture contents were being maintained experimentally.

ANALYSIS OF SYMPTOMS AS A KEY TO PATHOGENESIS

The writer pointed out earlier (7) that, in the Fusarium wilt of peas, wilting is not the most characteristic symptom and may, indeed, be completely absent when this disease assumes more the nature of cabbage yellows than of such wilt diseases as of tomato and flax. Whether death of the plant comes with wilting or with slow necrosis of the leaves, it is preceded by a series of conspicuous and diagnostically important changes which are significant indications of a disturbed physiology of the plant. Peas grown under conditions favoring early development of the disease exhibit the preliminary symptoms indicated in figure 2, A. Dwarfing results first chiefly from a reduced length of internodes and petioles but later, also, from retardation and then cessation of growth from the apical bud. Stipules and leaflets, reduced in size, become conspicuously rolled and distorted. The lower internodes of the stem increase in diameter and the entire shoot becomes more rigid, apparently from increased turgidity. The normal color gives way to slight yellowing of the leaves and to a superficial grayness suggesting an excess development of waxy bloom. Figure 2, B, illustrates the accompanying depression of root development.

Realizing that any attempt to understand the process of pathogenesis must have as a basis a clear picture of the disturbances in physiology and structure which constitute the disease, an attempt has been made to define more exactly the nature and extent of some of these changes.

For these studies, Badger variety peas were grown in deep flats of unsterilized clean soil and of infested soil that had been prepared by inoculation with the wilt fungus after sterilization several months before. They were grown in the greenhouse, where the temperature fluctuated between 18° and 25° C. With the few exceptions noted, diseased plants were se-

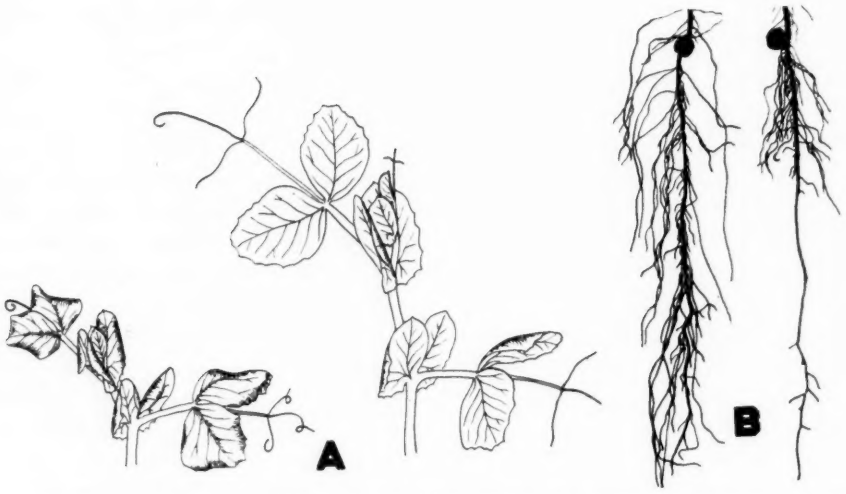

FIG. 2. A. Symptoms which precede wilting in the Fusarium wilt of peas. At the right is the upper portion of a healthy Badger pea seedling in the 7-node stage. At the left is a corresponding portion of a plant of the same age grown in wilt-infested soil and showing an extreme development of the characteristic symptoms which precede wilting in young plants. Note the shortened internodes and petioles, the dwarfed leaf laminae, the backward rolling of the lateral margins of the stipules, and the irregular distortion of the leaflets. Drawn from nature. B. Root dwarfing, a symptom of pea wilt. The root system at the left is typical of plants of the Badger variety grown 23 days from seed in the greenhouse in a 10-inch depth of clean soil. That at the right is of a plant of the same age and variety grown in wilt-infested soil. The shoot of this latter plant was wilting at the time this record was made, but there had been no appreciable root decay in either plant. Drawn from a photograph.

lected in which the preliminary symptoms were far advanced but before leaf necrosis or wilting had begun.

RATE OF DRYING OF HEALTHY AND DISEASED TISSUES

The writer observed earlier (7, p. 7) that diseased pea plants, when removed from the soil, wither more slowly than do healthy plants. This observation was tested quantitatively in the following comparison of rates of water loss from stem segments exposed to drying. Samples for this determination were each composed of 10 pieces of stem cut to the uniform length of 15 mm. Samples were prepared in duplicate from the aerial and subterranean portions of diseased and healthy plants, 28 days old from seed. In taking these samples, a block of stem, 5 mm. long, was cut away from the transition zone before the 15-mm. pieces were taken from the adjoining aerial and subterranean regions. Each sample was placed immediately in a dried and weighed weighing bottle with fitted stopper. With the aid of a

vernier caliper, measurements of stem diameter were made near the ends of every sample piece before the stems were cut.

Each bottle containing its sample was weighed and then its contents were spread to dry upon a fine wire screen, supported 14 inches above the table top, in a dark room where the temperature remained within the limits of 26.6° and 27.7° C. After 2 hours exposure the samples were returned to their bottles and weighed, after which they were again spread to dry. Alternate drying and weighing then proceeded until all samples had come to constant weight, the periods of drying being lengthened as the rate of water loss declined. Figure 3 presents graphically the results of this determination, each curve representing the average of duplicate samples which checked closely throughout.

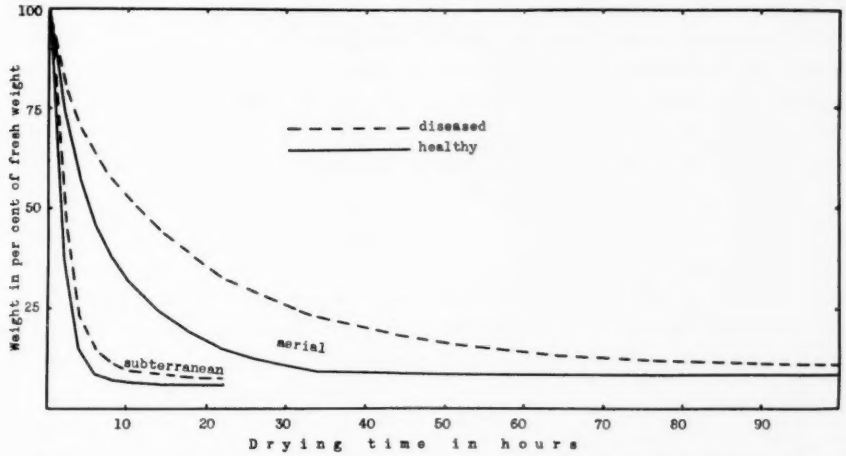

FIG. 3. Comparison of rates of drying of aerial and subterranean stem samples of diseased and healthy pea plants when exposed to the dry air of a constant-temperature dark room. Each curve represents the average of duplicate samples of 10 pieces of stem each, prepared as described in the text.

The healthy stem samples, both aerial and subterranean, lost water much more rapidly than the diseased and came to equilibrium with the air of the drying room in approximately half the time. The greatest contrast, however, came in the rapid drying of subterranean as compared with aerial samples, both healthy and diseased. This suggested that part of the difference between the healthy and diseased might lie in a different condition of the epidermal covering. A less detailed comparison was therefore made in which the sample pieces were split lengthwise before exposure. These lost water much more rapidly than did the unsplit, but still a striking difference between healthy and diseased pieces indicated that at least part of the water retentiveness results from a direct change in the internal tissues

of the stem. Comparative determinations indicated that leafy shoots dried to equilibrium less rapidly than the stem samples but showed a similarly great difference between diseased and healthy plants.

DRY MATTER AND ASH CONTENT

After these samples had reached equilibrium with the air of the drying room, they were dried to constant weight in the oven at 110° C. They were then transferred to baked-porcelain crucibles and incinerated to constant weight in an electric furnace. All the data are presented in table 3.

Striking differences between diseased and healthy plants are shown by these data with respect to the several points compared. In cross-sectional area the diseased aerial stems were 22.7 per cent greater than the healthy; in weight they were 44.5 per cent greater; and in computed specific gravity they were 13.5 per cent greater than the healthy. Consistently, the diseased plant samples showed a larger percentage of dry matter than did the healthy, the difference being greater in the subterranean samples (52.7 per cent) than in the aerial (25.9 per cent). Ash was almost directly proportional to dry matter, amounting to about 11.0 per cent of the oven-dry weight in all samples. It was thus more abundant on a fresh-weight basis in the diseased plants. The diseased stems not only contained a smaller percentage of water in the fresh condition but they retained more of it in the air-dry condition than did the healthy stems. Hygroscopic water amounted to 6.1 per cent of the dry weight in the healthy aerial stems compared with 8.7 per cent in the corresponding diseased stems.

OSMOTIC VALUE OF DISEASED AND HEALTHY TISSUES

The above data as well as evidence from histological studies suggest that important changes in cell contents throughout the plant are involved in the development of this disease. To test this further the plasmolytic method was employed for a comparison of the osmotic value of cell sap in healthy and diseased stems. The plants used were grown as indicated above but were in later stages of the disease and showed abscission or wilting of parts of some but not of all leaves. For comparison a few plants were taken in other stages of the disease. The tissue employed was the outer one or two layers of green subepidermal parenchyma from the second internode of the stem. This was chosen because of the ease with which suitable plastid-bearing cells were obtained from a uniform position by stripping off a portion of the epidermis with bits of adherent cortical tissue. Test solutions were prepared by dilution with double-distilled water from a molar solution of sucrose, and the actual tests were made in hollow-ground microscope slides. A separate plant was used for each of

TABLE 3.—*Comparison of stem size and of dry matter and ash contents of healthy Badger variety pea plants and of plants affected with the Fusarium wilt. All figures for weights, in milligrams, are average readings of duplicate samples of each of 10 pieces of stem. Figures for area and volume, in square millimeters and cubic millimeters, respectively, are averages based upon the single piece of stem as the unit*

	Samples of aerial stem				Samples of subterranean stem			
	Healthy	Diseased	Difference		Healthy	Diseased	Difference	
			Amount	Per cent			Amount	Per cent
Area of epidermis	111.0	123.6	12.6	11.3	114.0	121.6	7.6	6.7
Area of two cut ends	8.8	10.8	2.0	22.7	9.2	10.5	1.3	14.1
Volume	65.7	83.8	18.1	27.5	69.1	86.3	17.2	24.9
Computed specific gravity	0.96	1.09	0.13	13.5	0.83	0.87	0.04	4.8
Weight of fresh sample	631.0	912.0	281.0	44.5	575.0	748.0	173.0	30.1
Air-dry weight	54.0	101.0	47.0	87.4	34.0	66.0	32.0	94.1
Percentage of fresh weight	8.6	11.1	2.5	29.1	6.0	8.8	2.8	46.7
Oven-dry weight	51.0	93.0	42.0	82.4	31.9	60.5	28.6	89.7
Percentage of fresh weight	8.1	10.1	2.1	25.9	5.5	8.4	2.9	52.7
Hygroscopic water, per cent	6.1	8.7	2.6	42.6	7.1	8.7	1.6	22.6
Weight of ash	5.7	10.1	4.4	77.2	3.7	6.6	2.9	78.4
Percentage of oven-dry weight	11.0	10.6	0.4	3.8	10.8	10.7	0.1	0.9
Percentage of fresh weight	0.91	1.10	0.19	20.9	0.64	0.88	0.24	37.5

the 70 test mounts examined in this study. The average figures for osmotic values thus determined are set forth below, together with the concentration percentages of the isosmotic sucrose solutions and the calculated equivalent osmotic pressures:

	Healthy	Diseased
Osmotic value at incipient plasmolysis in terms of molar sucrose solution	0.37	0.54
Percentage concentration of sucrose in the isosmotic solution	12.7	18.5
Osmotic pressure, in atmospheres, of isosmotic sucrose solution at 22° C.	8.96	13.08

These average figures indicate an osmotic value 46 per cent higher in the diseased than the healthy plants. Of the healthy individuals tested none showed plasmolysis in 0.3 molar sucrose, while all plasmolyzed in 0.38. A few young plants gave plasmolysis in 0.35 and 0.36 molar solutions. Of the diseased plants, none gave plasmolysis in 0.53, but all did in 0.60 molar and stronger solutions. Practically all affected plants in medium and medium-late stages of the disease reacted similarly, but plants which showed very early symptoms and those that already had withered back to the succulent stem base commonly gave plasmolysis in solutions somewhat less concentrated than 0.53 molar. No evidence of extreme permeability of the cell membranes was detected in these determinations. Cells remained plasmolyzed without apparent change in the sugar solutions for 10 minutes or longer but recovered promptly when the solution was diluted or replaced with water.

REGENERATION OF AFFECTED PLANTS

When conditions prevent the rapid development of the disease, dying plants commonly produce one or more lateral shoots from their basal nodes. As shown in figure 4, such shoots may develop after the primary stem is almost dead and may remain healthy after it is completely withered. A number of sprouts may arise successively and grow to a height of several centimeters before each in its turn collapses. In the rapid development of the disease such sprouting may be absent or may be reduced to the formation of a few short stems which soon wither.

Regenerative ability is shown also by the experiment summarized in table 4 on the rooting of cut stems in water. Perfection variety peas, 26 days old from seed, some healthy from clean soil and others in early and medium stages of the disease, were washed from the soil, cut under water just above the cotyledons, and placed in culture tubes of tap water. These were held 8 days in the greenhouse before the data were taken. All the

FIG. 4. Sprout from the basal internodes of a pea plant affected with the Fusarium wilt disease. The diseased plant on the right has undergone progressive withering of leaves up to the apical bud without a distinct wilt phase and has sprouted from the second node, as is characteristic of the slow development of this disease. The cross (x) represents the height to which the fungus extended in the primary shoot as determined microscopically. This sprout showed no symptoms of the disease. Horsford's Market Garden variety peas grown 47 days in sparingly inoculated soil in a cool greenhouse where the temperature fluctuated between 14° and 20° C.

TABLE 4.—*Production of adventitious roots and axillary shoots by healthy and diseased pea stems in a period of 8 days*

Plant number	Stems from healthy plants				Stems from diseased plants			
	Adventitious roots		Axillary shoots		Adventitious roots		Axillary shoots	
	Number	Total length	Number	Total length	Number	Total length	Number	Total length
		Mm.		Mm.		Mm.		Mm.
1	3	72	0	0	10	367	0	0
2	3	36	2	12	12	330	0	0
3	4	36	0	0	9	128	1	23
4	0	0	0	0	13	201	1	37
5	2	3	0	0	3	35	1	22
6	3	72	0	0	9	95	1	12
7	2	4	0	0	...	.....	...	.....
Average	2.4	31.9	0.3	1.7	9.3	192.7	0.7	15.7

diseased plants, with the exception of number 5, which, initially, was in a more advanced stage of the disease, developed a larger number of roots and a greater combined root length than any of the healthy plants. Shoots appeared on 4 of the 6 diseased and on only 1 of the 7 healthy plants. Moreover, in 4 of the diseased plants, growth of the apical bud was resumed with the formation of new leaves free from rolling and distortion.

DISCUSSION OF SYMPTOM STUDIES

These various data and observations indicate clearly that the wilting or death of an affected pea plant is preceded by a series of profound disturbances in metabolism and growth which cannot well be ignored in future studies of this and related diseases. Theories of pathogenesis that have been focused chiefly upon the process of wilting and that have stressed the possible diminution of water supply to the leaves appear completely inadequate, for the actual wilting occurs, in this disease at least, as a relatively unimportant culmination of a long series of changes that are in no way simulated by experimentally-induced drouth. It seems probable that both the hypertrophy of parenchymatous cells and the increased rigidly of affected plants are attributable to the increased osmotic value of the cell contents which results, in turn, from accumulations of soluble reserves. In part, however, the rigidity is due to an increased thickness of cell wall and an abnormally early maturation of the skeletal tissues of the shoot. The marked increase of organic as well as inorganic reserves in diseased plants, together with the regenerative ability of such plants, suggests that the symptoms considered here do not result from a general starvation of the plant as a whole. Either the synthesis of abnormal materials poorly suited to the growth needs of the plant or an interference with the utilization of normal synthetic materials for growth is suggested. Failure of translocation is indicated particularly by the sprouting of affected plants from their basal nodes after apical growth of the primary axis has stopped.

The suspicion that a carbohydrate-high condition was indicated was not justified when graded applications of calcium nitrate were without apparent effect until applications were used that dwarfed even the check plants in noninfested soil. It is suggested that chemical analyses of the reserves accumulated in diseased plants might yield a more definite clue to the nature of the basic physiological disturbances involved.

WILTING INDUCED BY TOXIC CULTURE FILTRATES

Following the demonstration by Hutchinson (6), Brandes (1), and others, that a liquid culture medium in which a pathogenic microorganism has grown will cause wilting when introduced into cut stems of the host

plant, considerable attention has been devoted to toxicity theories of the pathogenesis of those wilt diseases in which the pathogene invades primarily the vascular tissues. Assuming that a fungus liberates the same toxic substances within the vascular system of its host plant as it does in a staling culture, a study of the wilting induced experimentally in cut stems by culture filtrates or fractions of such filtrates should provide a key to the nature of wilting and of the toxic substances involved in pathogenesis.

Haymaker (3) has presented evidence recently in support of the view that such studies are applicable to the problem of resistance as well as of pathogenesis. Working with the Fusarium wilt of the tomato he was able to simulate the symptoms of the disease more closely than others have done, including some aspects beyond mere wilting and collapse of leaves and stems. Moreover, he found a direct correlation between degrees of pathogenicity of *Fusarium lycopersici* and toxicity of its metabolic products. Culture filtrates were more toxic when the fungus was grown at the optimal temperature for pathogenesis than when grown at other temperatures. Filtrates from cultures of highly pathogenic strains of the fungus were more toxic than those from weakly pathogenic strains. And, of special interest in this present consideration, he demonstrated with many varieties of tomato what White (14) had found with two, that varieties, resistant to the wilt disease, are less readily damaged by these culture filtrates than are the susceptible varieties.

EXPERIMENTAL STUDIES

To determine the applicability of these findings to the problems of pathogenesis and resistance in pea wilt, the writer has used chiefly the technique of placing cut pea stems into filtrates from solution cultures of the wilt fungus and observing the wilting or other injury that resulted. Pea seedlings were grown in 12-inch clay saucers of autoclaved soil in the greenhouse where the temperature varied between 19° and 25° C. All saucers were kept under the same conditions on the same bench. Seedlings were used at the age of 20 to 23 days in the different experiments. They were washed from the soil to avoid breaking; the roots were washed clean; and each stem was cut off under water with a razor blade at a point just above the cotyledonary node. The cut stems were then rinsed in boiled water and transferred to the test solutions.

For the toxic culture filtrates, strain 182c of *Fusarium orthoceras* var. *pisi* was grown in White's modification (14) of Richards' Solution,[4] with 250 cc. in each liter flask. Cultures were incubated 15 to 22 days in the different series at room temperature, 20° to 24° C. The filtrate was then

[4] KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mg.; dextrose, 50 gm.; and water, 1,000 cc.

TABLE 5.—*Varietal susceptibility to the toxic action of filtrates from Richards' solution cultures of the pea-wilt fungus, Fusarium orthoceras var. pisi, and of solutions of the alcoholic precipitate from such filtrates*

Varieties susceptible (S)[c] or resistant (R)[c]		Index of wilting[a] after 24 hours in the indicated dilutions of the following solutions											Index of flagging[b] after 19 hours in the indicated molar solutions of dextrose			
		Filtrate from Richards' Solution cultures of the pea-wilt fungus								Solution of alcoholic precipitate from filtrate						
		Trial I				Trial II										
		F/1	F/2	F/4	Total	F/2	F/4	F/8	Total	APS/1	APS/2	Total	0.8	0.6	0.4	Total
Badger	(S)	10	7	4	21	6	3	0	9	2	0	2	9	0	0	9
Horsford	"	9	7	5	21	5	3	1	9	4	0	4	9	6	0	15
Advancer	"	10	8	8	26	7	4	2	13	10	1[d]	11[d]	15	11	0	26
Perfection	"	10	4	3	17	6	4	2	12	2	0	2	9	9	0	18
Horal	(R)	10	9	6	25	7	5	1	13	3	1[d]	4[d]	3	0	0	3
Rogers' K	"	10	8	6	24	7	5	2	14	4	0	4	15	15	11	41
Green Admiral	"	6	6	4	16	5	2	0	7	2	0	2	15	15	0	30
White Admiral	"	10	9	5	24	5	4	2	11	5	0	5	15	15	6	36

[a] Index of wilting based upon the following values: Leaves and upper third of stem fully withered = 5; leaves withered but stem scarcely affected = 4; all leaves withered but bud and petioles scarcely affected = 3; some but not all leaves fully wilted or withered = 2; and some leaves wilting but none fully withered = 1.

[b] Index of flagging based on the following values: Stem fully shriveled at base and leaves far wilted = 5; and plant flaccid but not shriveled = 3.

[c] As determined by field and greenhouse tests in which the seed is planted in infested soil. Resistance and susceptibility are sharply defined under these conditions.

[d] One plant was lost during the experiment, otherwise this figure might have been greater.

prepared by repeated passage through fine filter paper and was diluted as needed by the addition of boiled distilled water. Controls consisted of boiled water and of sterile Richards' Solution, filtered and diluted as indicated.

Preliminary experiments with this technique indicated that both resistant and susceptible varieties of peas will wilt when treated in this way. A more extensive experiment was then conducted, as indicated in table 5, trial I, to compare in detail the responses of resistant and susceptible varieties. Seedlings of 8 varieties, 21 days old, were prepared as indicated above and placed singly in duplicate culture tubes of water, of sterile Richards' Solution, and of filtrate from 15-day cultures, the latter two in three concentrations; full-strength, half-strength, and quarter-strength. All plants in water and in the diluted Richards' Solution (Fig. 5, plant 5) remained fresh and turgid. In the full-strength Richards' Solution a general flagging (Fig. 5, plant 4), very different from wilting, was observed in all varieties except Green Admiral (R)[5] and Perfection (S),[5] which

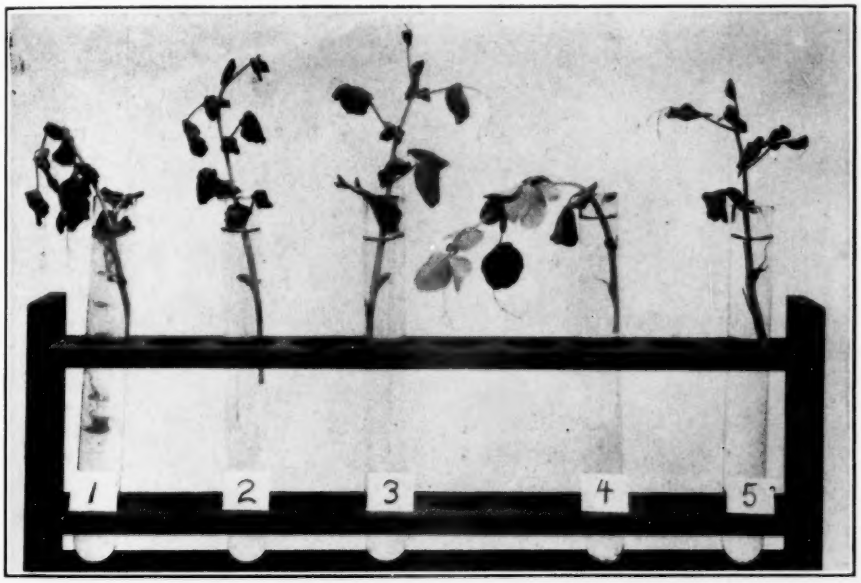

FIG. 5. Wilting of the disease-susceptible Horsford variety in stale culture filtrates and flagging in Richards' Solution. These excised stems were photographed after 19 hours in solutions as follows: 1, full-strength culture-solution filtrate; 2, half-strength filtrate; 3, quarter-strength filtrate; 4, full-strength sterile Richards' Solution; and 5, quarter-strength Richards' Solution. Note the distinction between wilting, as shown by plants 1, 2 and 3, and flagging, as shown by plant 4. See trial I, table 5.

[5] (R) and (S) represent resistant and susceptible varieties, respectively. See footnote [c], table 5.

remained turgid. Wilting occurred in the culture filtrates in all varieties in proportion to the concentration of filtrate. Furthermore, the 8 varieties differed considerably among themselves in their susceptibility to injury, as is seen by inspection of the column of totals under trial I. While the highest index of wilting[6] observed, 26, was for the susceptible variety Advancer, and the lowest, 16, for the resistant Green Admiral, the total of the four indices for the disease-resistant varieties was slightly greater than for the susceptible varieties. In other words, while there were distinct differences in the reaction of the several varieties, there was no apparent relationship between this and resistance or susceptibility to the wilt disease.

FIG. 6. Pea shoots wilting in stale culture filtrates after 20-hours exposure. See trial II, table 5. A. Type of injury observed in the disease-susceptible variety, Advancer. The plant at the left has stood in half-strength filtrate from a 22-day culture of the pea-wilt fungus in Richards' Solution; that at the right in half-strength sterile Richards' Solution. B. Comparison of wilting in the disease-resistant variety Horal (left) and the disease-susceptible Badger (right) in half-strength filtrate from a 22-day culture.

[6] See footnote [a], table 5.

In another experiment, trial II of table 5, 20-day seedlings of the same 8 varieties were tested with filtrates from 22-day cultures. To avoid flagging and to retard wilting, full-strength filtrates and Richards' Solution were omitted and $\frac{1}{8}$-strength solutions were added. Figures 6, 7 and 8 illustrate some of the test plants after 20 hours. In water and in the diluted Richards' Solution, all plants of all varieties remained turgid after 24 hours. In all dilutions of filtrate, all showed some injury with the exception of 2 varieties in the greatest dilution. Wilting, again in this experiment, was proportional to the concentration of the filtrate and varied from one variety to another but was not related to the resistance or susceptibility of these varieties to the wilt disease. For example, figure 6, B shows distinctly greater injury in the Horal (R) than the Badger (S), and figure 7, comparing 2 resistant varieties, shows much greater injury in Rogers' K than in Green Admiral. In this trial the total index of wilting was again slightly greater for the 4 resistant than the 4 susceptible varieties.

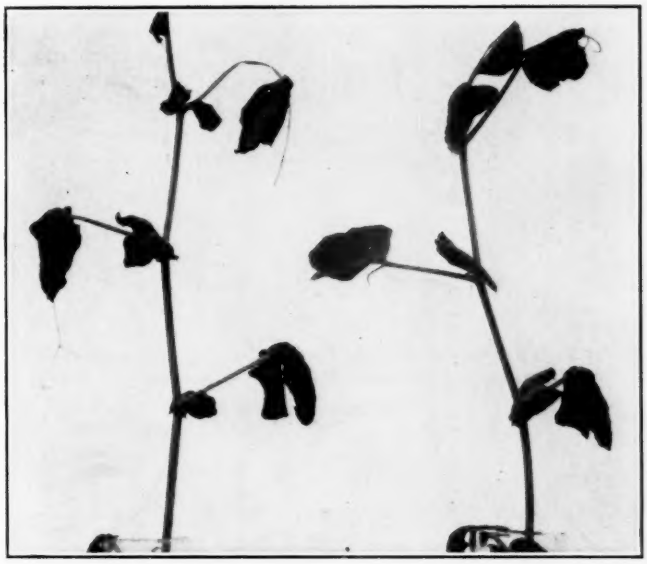

FIG. 7. Comparison of wilting in two disease-resistant varieties in stale culture filtrates. These plants, Rogers' K at the left and Green Admiral at the right, have stood 20 hours in half-strength filtrate from a 22-day culture. See trial II, table 5.

Since this observed absence of correlation between resistance to disease and resistance to toxic wilting was directly opposed to the findings of Haymaker (3) for tomato, another test was made using only the alcoholic precipitate fraction instead of the entire filtrate. Clear filtrates from 20-day cultures were precipitated with 2 volumes of 95 per cent alcohol,

TABLE 6.—*Eight varieties of peas listed in the descending order of their susceptibility to wilting under the conditions indicated, their susceptibility to flagging in dextrose solutions, and the relative heights of their shoots at the time of testing. Two or more varieties within brackets are of equal rank. Compare with table 5*

Susceptibility (S) and resistance (R) to Fusarium wilt	Susceptibility to wilting from toxic action			Susceptibility to flagging in graded solutions of dextrose	Relative heights of varieties at time of testing
	Filtrate from culture of wilt fungus in Richards' Solution		Aqueous solution of alcoholic precipitate		
	Trial I	Trial II			
(S) Advancer, Perfection, Badger, Horsford	Advancer (S) Horal (R) Rogers' K (R) White Admiral (R) Badger (S) Horsford (S)	Rogers' K Advancer Horal Perfection White Admiral Badger Horsford	Advancer White Admiral Horal Rogers' K Horsford Badger Perfection Green Admiral	Rogers' K White Admiral Green Admiral Advancer Perfection Horsford Badger Horal	Rogers' K White Admiral Green Admiral Advancer Horsford Badger Perfection Horal
(R) Horal, Green, Admiral, White, Admiral, Rogers' K	Perfection (S) Green Admiral (R)	Green Admiral			

washed with alcohol, dried, and then digested 2 hours at room temperature with $\frac{2}{3}$ the original filtrate volume of distilled water. The solution was then filtered and diluted for use and was tested in duplicate with 23-day plants of all 8 varieties. This solution proved less toxic than the whole filtrate and produced no visible injury in dilutions greater than half-strength in the 24-hour period. Even at the full strength the injury produced was relatively slight, as shown in table 6, and yet again there were distinct differences in varietal behavior that were not correlated with the susceptibility of these varieties to the Fusarium wilt.

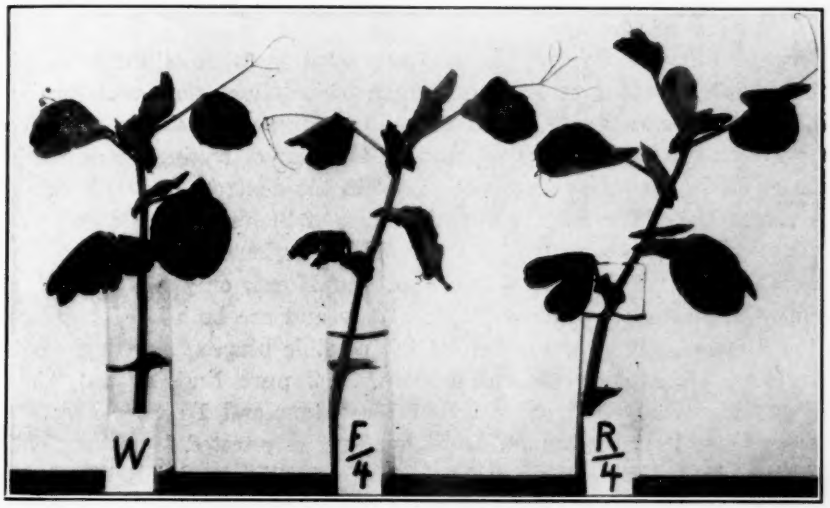

FIG. 8. Wilting of the disease-susceptible variety Advancer in stale culture filtrate. These plants have stood 20 hours in (left) distilled water, (center) quarter-strength filtrate from a 22-day culture, and (right) quarter-strength Richards' Solution. Note that the water lost from these tubes, chiefly through transpiration, is much greater in the case of the culture filtrate, where wilting occurred, than in the Richards' Solution, where all leaves remained turgid.

In trial I the 2 varieties least damaged by the filtrates, Green Admiral and Perfection, were the only 2 that did not flag in the full-strength Richards' Solution. This suggested that some difference in osmotic values might account for the observed varietal differences in susceptibility to injury. Accordingly, after a preliminary trial to determine suitable concentrations, cut stems of 15-day plants were placed in triplicate in the following solutions of dextrose: 0.4, 0.6, and 0.8 molar. After 19 hours the index of flagging was computed as indicated in footnote ([b]), table 5, as a numerical basis for comparison of the varieties. The varietal differences thus revealed are much greater than in the toxicity tests but show no apparent correlation with either toxic injury or the wilt disease.

For more ready comparison the results of these several tests have been summarized in table 6, where the 8 varieties are listed in the descending order of their susceptibility to injury in each of these tests and in the descending order of stem length at the time of testing. Comparison of these listings reveals a general agreement between trials I and II and the alcoholic precipitate test, in spite of disagreement in detail, but shows a complete lack of agreement between these listings and the resistance or susceptibility of these varieties to the wilt disease. In trial I, 3 of the 4 most damaged varieties were resistant to the disease; in trial II, 2 of the first 4 in the list are resistant; and, in the alcoholic-precipitate test, 3 of the first 5 are resistant.

Susceptibility to flagging in dextrose solutions throws these varieties into an entirely different order of rating, which shows no correlation with either resistance to the disease or to toxic action but which correlates very closely with the heights[7] of the plants at the time of testing. From this it appears likely that the collapse observed in the dextrose solutions is not a direct measure of the relative osmotic values of the test plants.

Injury in the toxic filtrates was not proportional to height of plant. Rogers' K, one of the tallest varieties, and Horal, only half as tall, are among the most susceptible to toxic injury and are both highly resistant to the disease. As a further test of the possible bearing of morphological characters, an experiment was made with 2 pure lines of the Alcross variety,[8] identical in appearance, but 1 resistant and 1 susceptible to the disease. Two 19-day plants of each pure line were tested for susceptibility to injury in 4 different concentrations of: (a) Richards' Solution; (b) filtrate from 17-day cultures filtered through paper alone; (c) filtrate from similar cultures filtered through paper and alundum cups; and (d) aqueous solutions of alcoholic precipitate from the paper filtrate (b). Observations over a period of 90 hours revealed no consistent differences between these strains of peas, in spite of the sharply drawn distinction in their reaction to the disease.

In a minor supplementary test, toxic culture filtrates were introduced through cut petioles into the stems of growing plants without disturbing the roots. After 1 week the plants thus treated showed various external symptoms, none wholly characteristic of the disease. Microscopic examination revealed no great disturbance in control plants where water alone was used, but, in both Horal (R) and Horsford (S), the introduction of culture filtrates had led to a limited amount of gumming of the vascular tis-

[7] Differences in height are the result chiefly of differences in length of stem internodes; in fact, 2 of the shortest of these varieties had slightly more nodes at a given age than 4 of the varieties of intermediate height.

[8] This seed was kindly furnished by Mr. Earl J. Renard.

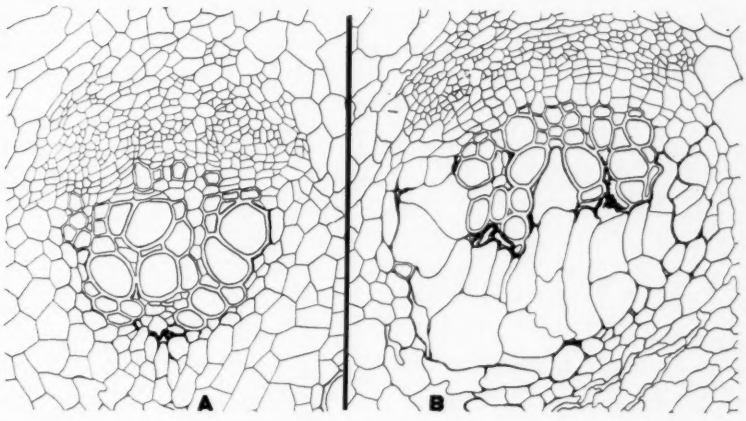

FIG. 9. Hypertrophy of xylem and adjacent parenchyma of a leaf trace in response to the introduction of filtrate from a 15-day Richards' Solution culture of the pea-wilt fungus into a Horsford pea plant through a cut petiole. Drawn with the aid of a camera lucida, ×173. A. Normal leaf trace in the fourth internode, a few millimeters below the cut petiole and on the opposite side of the stem. B. The affected leaf trace, drawn from the same section as A. Note the extreme hypertrophy of parenchyma between and around the xylem vessels; the more moderate hypertrophy of the cambium and metaphloem region; the collapse of some of the protoxylem; and the crushing of thin-wall pith parenchyma.

sues and to a conspicuous hypertrophy of xylem and surrounding parenchyma in and around the stipule traces and leaf traces involved (Fig. 9). The presence of pieces of mycelium in these leaf traces makes it impossible to attribute all of this disturbance to the toxic substances introduced; yet the promptness with which the hypertrophy occurred in proportion to the small amounts of living fungus present suggests that the response was chiefly to the filtrate itself. No check with sterile Richards' Solution was used. The similarity of this to the hypertrophy of cambium and stelar parenchyma in the development of the pea-wilt disease (7) suggests that this type of study merits further application in the testing of toxic culture filtrates.

DISCUSSION OF TOXICITY

From these studies it is evident that resistance to the Fusarium wilt of peas is not a question of resistance to staling products of the wilt fungus. This is directly contrary to the finding of Haymaker (3) with respect to resistance to the Fusarium wilt of tomato.

The type of injury produced by toxic culture filtrates did not simulate closely the symptoms of pea wilt, but this was not expected. Symptoms of this disease require for their development a period of several days during

which a small but increasing effect of the fungus must be operative, and the conditions imposed by this technique made this impossible. The wilting observed in these experiments did, however, appear as closely comparable to the collapse of diseased plants as might be expected in the absence of the preliminary changes in the plant typical of the disease.

In spite of the criticisms of Hursh (5) and others, this general technique appears to be valid, and extremely useful in studies of some aspects of pathogenesis. Clearly, this wilting was a result or accompaniment of collapse of the cells of, first, the leaves and, then, the stems under the influence of toxic substances in the filtrates and not a consequence of a diminished supply of water to the leaves. Unlike the conditions described by Haymaker (3) for the tomato and illustrated by Hursh (5), this toxic injury did not first involve a general flaccidity of the succulent parts of the plant except when the strongest solutions were employed. With the more dilute solutions the affected leaflets or their margins first became water-soaked in appearance and dark green in color, then lost their turgor, withered, and dried. Collapse of petioles and stem proceeded only after leaflet collapse, as shown by figures 6, 7 and 8, a very different sequence from that observed in the flagging of plants from lack of sufficient water in strong Richards' Solution (Fig. 5, plant 4) or in the dextrose solutions.

As appears from transpiration records (8) to be the case in the wilting of diseased plants, wilting of these cut stems was accompanied by rapid loss of water. This question arose after the experimental work was concluded, but examination of the writer's extensive photographic records of the experimental plants showed repeatedly, as indicated in figure 8, that more water was lost during the experimental period by the wilting plants than by healthy plants in the corresponding concentrations of Richards' Solution. The osmotic value of sterile Richards' Solution was probably nearly the same as that of the toxic filtrate. White (14, p. 229) found no significant difference in a similar case. It is clear, then, that the wilting plants in toxic solutions transpired or lost by evaporation from necrotic surfaces considerable amounts of water. The toxic substances were acting directly upon the leaf cells and not, as suggested by Hursh, primarily damaging the vascular system of the stem. This is indicated further by the fact that Horal and Rogers' K varieties, one only half as tall as the other, reacted in the same way in spite of different lengths of vascular system, the reaction being primarily in the leaves. This technique has not simulated the late wilting of large plants, which may sometimes involve a diminished supply of water to the leaves.

GENERAL DISCUSSION

The course of development of the pea wilt disease is more varied than might perhaps be implied from the foregoing considerations. The dis-

ease results from the interaction of one pathogenic fungus with different varieties of the host-plant species under diverse conditions, as of nutrition, temperature, and moisture; and both the course and end point of the interaction vary with the conditions. The course of the disease set forth here is typical for conditions which favor the rapid development of the disease in young plants. When, however, conditions are less favorable or when the host is approaching maturity at the time symptoms first appear, the sequence of events is different. Throughout this variation wilting is not the most constant symptom, for it may be completely displaced by a slowly progressing but none the less complete collapse. This suggests therefore that wilting merits proportionately less attention than that given it by some investigators and that the causes of necrosis, whether slow or rapid, be given more consideration; particularly, since in this disease the characteristic wilting appears to differ from slow necrosis, chiefly in rate of progress.

Wilting in older plants may be a different phenomenon. In pea plants that are half grown or larger when the disease develops, symptoms are more nearly those of the conventional wilt disease than those described in this paper. Preliminary symptoms may be much reduced; particularly, in plants that have attained most of their stem growth, but they can be detected even in nearly mature plants. It is in such large plants that indications of alternate wilting and recovery have been noted. Necrosis is sometimes less intimately associated with this late wilting; and thus there is the suggestion that a diminished supply of water may stand in a causative relationship, particularly, since microscopic examination of the taproot and stem base of such plants commonly reveals a great abundance of the fungus in the xylem vessels. While this mycelium, in itself, probably does not greatly hinder the passage of water, still, through the liberation of carbon-dioxide as suggested for *Fusarium lini* by Tochinai (12), it might effectively obstruct the passages. However, even in the older plants, symptoms which precede wilting indicate a gradual disturbance of synthetic or assimilative processes of the host; and the close association of necrosis with wilting sometimes observed in such older plants suggests that along with a diminishing supply of water there is progressively increasing yield of toxic metabolic products being carried up to the leaves from the increasing quantity of mycelium, and that such toxic substances are operative along with shortage of water in causing the collapse.

In younger plants, wilting and necrosis appear to be the culmination of a series of important changes in metabolism and growth of the host brought about by the fungus through the action, apparently, of products of its own metabolism. The possibility of toxic products being liberated by damaged host cells, as pointed out by Overton (10) and Haskell (2), must

be admitted. That such collapse is not a result of general starvation is shown by the accumulation of organic and mineral reserves and by the regenerative ability of affected plants. This does not prove, however, that local starvation, through failure of translocation, or unbalanced starvation through unfavorable utilization or synthesis, may not be significant. Moreover, as shown in a foregoing paper (8), water is lost at an apparently increased rate during rapid collapse of young plants. This further supports the view that the total injury is a result not of lack of water but rather of a complex and systemic toxic action which causes death of the leaves and stem only after a long series of disturbances in the metabolic and growth balance of the plant.

Resistance, inherent in some varieties of peas, appears to lie either in obstruction of entry of the pathogene into the vascular system of the pea plant or in resistance to its growth after such entry. Contrary to the expectations raised by the work of White (14) and of Haymaker (3), resistant varieties of peas, as a group, are no more tolerant of toxic culture filtrates than are the susceptible varieties. Other aspects of resistance are considered in following papers.

SUMMARY

This paper is one of a series reporting studies of pathogenesis and resistance in the wilt of peas caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford carried out as an incumbent of a National Research Council Fellowship in the Biological Sciences. Histological investigations are being reported separately.

The pea-wilt fungus can infect the pea plant and produce the disease without the aid of other microörganisms or of gross mechanical injury. Peas grown in cotton-plugged culture tubes of sterilized soil or of an agar substratum inoculated with a pure culture of the pathogene develop the symptoms readily. Wounding the root system delays slightly, rather than hastens, the appearance of symptoms in plants of a susceptible variety but does not lower the degree of resistance of resistant peas.

Pea wilt develops more rapidly in soil heavily infested with the wilt fungus than in lightly inoculated soil. Dilution of highly infective soil with steam-sterilized soil leads to greater retardation of the disease than a corresponding dilution with noninfested raw soil.

A search for soil toxicity developed by the action of the wilt fungus has given only negative results.

In the characteristic development of pea wilt under conditions favorable for the disease, there are very distinctive and significant changes in the affected plants which precede wilting or leaf necrosis. The course and degree of some of these changes are considered to provide a key to probable

factors in pathogenesis. Pronounced dwarfing is accompanied by increased rigidity of the entire shoot, hypertrophy of the lower stem internodes, and rolling of leaf laminae. Before wilting begins, diseased pea stems lose water more slowly upon exposure to drying and have a higher content of dry matter with a proportionately increased ash content, an increased osmotic value of the cell sap, and an increased capacity for regeneration. These symptoms do not indicate general starvation or drouth of the plant as a whole but they do suggest unbalanced nutrition and failure of translocation.

Filtrates from Richards' Solution cultures of the wilt fungus produce a type of rapid necrosis in cut pea stems which appears comparable to the sudden wilting of young plants in the early development of this disease. Varieties of peas differ in susceptibility to toxic culture filtrates, but experiments with 10 strains and varieties have failed to reveal any correspondence between this and the resistance or susceptibility of these same varieties to the Fusarium wilt.

A shortage of water induced directly or indirectly by the fungus may sometimes be a factor in the wilting of large plants but probably not in the early collapse of young seedlings. In the main, pathogenesis must be attributed to the action of toxic substances resulting from the presence of the wilt fungus within the host plant; but this is a far more complex and systemic action than generally conceived, leading first to a derangement of metabolic and growth processes and finally to death of the tissues. Actual wilting or slow necrosis, as the case may be, comes as the culmination of a long series of changes induced by the influence of the fungus.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

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WOUND INOCULATION IN RELATION TO RESISTANCE IN THE FUSARIUM WILT OF PEAS[1]

MAURICE B. LINFORD

INTRODUCTION

In the course of histological studies of resistance and susceptibility of peas (*Pisum sativum* L.) to the wilt disease caused by *Fusarium orthoceras* App. and Wr. var. *pisi* Linford, it became desirable to gather experimental evidence concerning the possible localization of the expression of resistance. The writer showed earlier (1) that varietal resistance to this disease is sharply defined, approaching very close to immunity even under conditions most favorable for the disease in susceptible varieties of peas. Wade (5) has since presented further evidence that inherent resistance is essentially complete. The writer's tests with toxic culture filtrates (2) showed that resistance in this case is not a question of tolerance by the resistant plants for the products of fungal metabolism. Preliminary histological investigations (1) had indicated that the fungus failed to establish itself in the vascular system of the resistant plants and suggested that resistance is expressed chiefly in the root system. It appeared probable that if the wilt fungus could be introduced directly into the vascular system of the aerial shoot by some mechanical means, the disease might develop in resistant as well as in susceptible plants. This was attempted through the inoculation of wounds in stems and petioles which exposed truncated vascular bundles to the pea-wilt fungus.

EXPERIMENTAL

Peas of the Badger (susceptible) and Horal (resistant) varieties, grown 10 days from seed to the 4-node stage in pots of sterilized soil, were inoculated as indicated in table 1. As soon as a plant was inoculated it was wrapped in wet absorbent cotton and gauze, and, when all plants in a pot were ready, additional cotton was applied; the whole was wetted thoroughly and placed in a moist chamber where it was kept wet for 40 hours. Bandages were then removed and the pots were placed in the open greenhouse for the remainder of the experimental period of 1 month. Fifteen plants of Badger and 15 of Horal were inoculated in each way, and 5 of each were

[1] This paper is one of a series reporting studies on pathogenesis and resistance in the Fusarium wilt of peas conducted under a fellowship appointment from the National Research Council. The writer wishes again to express his appreciation to the Department of Botany, University of Wisconsin, for the facilities extended, and to Professor B. M. Duggar for helpful criticism throughout this work.

TABLE 1.—*Results of wound inoculation experiment with Fusarium orthoceras var. pisi and both wilt-resistant and susceptible varieties of peas. Data taken after 1 month*

Method of inoculation	Badger variety (susceptible		Horal variety (resistant)	
	Number infected[a]	Per cent	Number infected[a]	Per cent
1. Tangential slice cut in cortex of second stem internode and barley-grain culture inserted	0	.....	1	6.7
2. First petiole excised at its base and barley-grain culture applied to wound	2	13.0	3	20.0
3. First petiole excised at its base and Richards' solution culture applied to wound	0	.....	0	.....
4. Stem tip removed above fourth node and barley-grain culture applied to wound	1	6.7	2	13.3
5. Not wounded. Barley-grain culture placed on soil	0	.....	0	.....

[a] Fifteen plants each of Bodger and Horal peas were inoculated by each method. Five plants of each variety were wounded and left as uninoculated controls. These controls all remained healthy. Details of technique are given in the text.

held as controls, with the plants wounded but not inoculated. Additional controls were provided by spreading inoculum on the surface of the soil around non-wounded plants and by the omission of all wounding and application of the fungus.

All the control plants remained healthy and recovered from the wounding without conspicuous reaction or loss of vigor. Of the inoculated plants a small percentage became infected, and these were all from the barley-culture inoculation. Of the 45 plants of each variety, 90 plants in all, inoculated through wounds with the culture growing on cooked barley kernels, 3 plants of the Badger variety, or 6.7 per cent, and 6 of the Horal, or 13.3 per cent, became infected as indicated by the development of symptoms.

The symptoms developed by these plants were closely similar to those typical of the wilt disease but differed somewhat both in details of appearance and in sequence of development. Response became apparent in

Badger and Horal alike a few days after inoculation and developed rapidly to its maximum expression, which varied from plant to plant. Growth of the terminal bud was retarded or completely checked, stems and petioles became swollen and rigid, and leaflets and stipules rolled backwards or became distorted, thick, firm, and darker green than normal. The whole plant assumed the condition of extreme rigidity, typical of this disease before wilting begins, but showed none of the general yellowing and the excess development of waxy bloom which accompany its development from natural infection through the roots. After this quick reaction, further changes occurred slowly. In some plants of both varieties, stipules, leaflets, and petioles, and even the stem tips collapsed in a manner typical of severe development of the disease. One plant of each variety made partial recovery, resuming apical growth with the production of approximately normal leaves. The other affected plants neither recovered nor developed more severe symptoms during the remainder of the experiment.

HISTOLOGICAL FEATURES

Microscopic examination of infected plants of both varieties revealed a condition different from the typical pathological histology of pea wilt but with many points of essential similarity. Free-hand sections and serial paraffin sections were prepared from the infected plants and from several healthy control plants. In the controls there was little vascular discoloration or plugging of the vessels with gum-like materials, and this only in or near the actual wounds. Inoculated plants which had not become infected showed similar but somewhat greater disturbances. In all plants which showed symptoms of the disease, however, the fungus was present abundantly in the margins of the wounds and extended variable distances into the connecting vascular bundles.

Even in the most severely damaged plants there was no general invasion of the vascular system. The fungus remained localized within those vascular bundles which it entered first and was apparently limited in its vertical extent by the occlusion of vessels with products of wound reaction. In the single plant infected from a cortical wound the fungus was found in the cortex and in both stipule traces; in plants inoculated through cut petioles it extended chiefly downward through the leaf trace; and in plants inoculated through the stem tip it extended downward along several bundles. In most plants it was limited to a vertical extent of a few millimeters from the wound, and in no case was it seen to have traversed more than two internodes from the point of inoculation.

Such local development of the fungus within a vascular bundle had stimulated the surrounding parenchymatous cells, leading to the formation

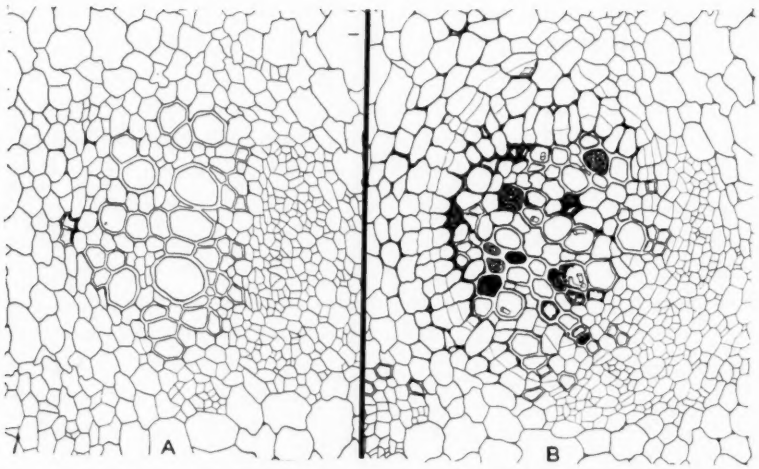

FIG. 1. Leaf traces of Horal (R) pea plant inoculated with the pea-wilt fungus through a cut petiole at the third node. This plant had stood one month following inoculation before the material was fixed for sectioning; the stem was swollen and the leaflets were all collapsed. Beyond the parenchyma immediately in the margin of the wound the fungus was present only in the leaf trace of the inoculated petiole. Drawn with the aid of a camera lucida, × 203. A. Leaf trace free from visible disturbance, from the upper part of the third internode, below the inoculated petiole but from the opposite side of the stem. B. Infected leaf trace from the same level in the third internode. This vascular bundle, which extends into the inoculated petiole, illustrates varied responses to invasion of the vessels, including accumulation of gum-like and granular deeply staining materials in the vessels; altered staining reaction of walls, particularly at the margin of the xylem tissues; the formation of a periderm-like sheath surrounding the xylem, through both hypertrophy and hyperplasia of the parenchyma; and a mild hypertrophy of meta- and secondary phloem.

of a cambium-like layer and the development of a sheath of new tissue several cells in thickness surrounding the xylem portion of the bundle. This occurred both in the central cylinder (Fig. 1, B) and around the stipule traces in the cortex (Fig. 2, B). In some instances these newly formed cells enlarged greatly, crowding the normal surrounding tissues markedly out of place.

It was demonstrated clearly in this study that even a small amount of the fungus within the plant is sufficient to produce symptoms of this disease. In the single Horal plant which became infected from inoculation through a cortical wound (Fig. 2) the leaflets, stipules, petioles, and even the tip of the stem wilted. Serial sections from a point well above the lesion to a point far below showed that in no place had the fungus entered the central cylinder. It was present only in the cortical parenchyma at the margin of the wound and in the two stipule traces through which it had

extended a few millimeters up and down the stem. In such a case it seems clear that the development of preliminary symptoms and finally the collapse of leaves and succulent parts of the stem were stimulated by the action of the wilt fungus at a distance, acting through some toxic substances which became distributed throughout the plant.

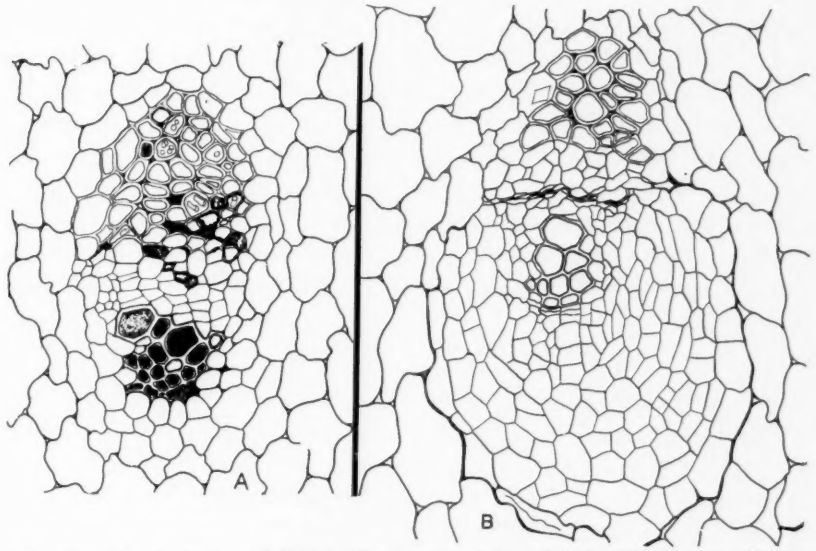

FIG. 2. Stipule traces of Horal (R) pea plant inoculated with the pea-wilt fungus through a cortical wound in the second internode. This plant had stood one month after inoculation and was in late stages of collapse at the time this material was fixed for sectioning, but infection was limited to these two stipule traces and the margin of the cortical wound. Drawn with the aid of a camera lucida, ×290. A. Occlusion of xylem vessels and invasion of phloem fibers of stipule trace just below the lower limit of cortical invasion at the margin of the inoculation wound. Except the one large vessel with granular contents, all vessels in this bundle were completely occluded with gum-like material at some level, although not all at one level. Invasion of the phloem and phloem fibers was accompanied by necrosis and an accumulation of deeply staining matter in and between the cells. Compare with the nonnecrotic condition of B. B. The other stipule trace from a section through a lower level of the same internode, showing a condition of extreme hyperplasia of the parenchyma surrounding the xylem. Closer to the wound this vascular bundle was largely occluded and still closer was thoroughly invaded by the wilt fungus. Note the cambium-like activity immediately at the base of the protoxylem. The metaphloem has been crushed by this hyperplastic tissue.

Occlusion of xylem vessels with brown, granular or more typically gum-like, deeply staining materials was a conspicuous feature of this infection and apparently was a major factor in the very limited longitudinal distribution of the fungus along the vessels. Gum-like material (Fig. 2, A) was present in the vessels not only in immediate association with the fungus

but also at a point well in advance. These materials, apparently secreted into the lumina of the vessels by the xylem parenchyma, appeared to constitute a barrier to the progress of this fungus. Near the wound where the fungus was abundant in the vessels, the xylem parenchyma cells were chiefly dead and wound gums were present only sparingly; farther from the wound, where the fungus was less abundant and the parenchyma still alive, such materials were relatively more abundant; and still farther away, beyond the fungus, they were seen to plug some or all the vessels of an infected bundle (Fig. 2, A). Substances of similar appearance were seen in and on the walls of the parenchyma cells damaged by the fungus, both cells of the primary tissues and cells formed in response to this invasion. As will be shown in a following paper, this appears directly comparable with some observed host reactions in the normal development of the wilt disease in susceptible plants. Likewise it resembles certain more pronounced responses observed in the tissues of resistant pea plants grown in infested soil.

DISCUSSION

The low percentage of infection obtained in this work is in agreement with the experience of Tisdale with flax wilt (4) and indicates that the pea-wilt fungus is not an aggressive invader of wounds. Tisdale, however, obtained no infection of resistant plants. The writer (2) and Wade (5) failed to find evidence of invasion of pea roots through wounds by the pea-wilt fungus. This is indicative of a specialized parasitism on the part of the fungus and also of a mild degree of resistance to the invasion of aerial parts even in the susceptible variety of pea. The pronounced host response, comparable with some resistance reactions, which apparently served in this case to check advance of the fungus, is further evidence that both varieties alike are resistant to the wilt fungus when it is thus introduced without any of the preliminary alterations in the host physiology which typify the development of pea wilt from the usual root infection (2).

The symptoms that developed in the infected plants were as closely similar to the characteristic symptoms of pea wilt as might be expected and were the only close approach to the symptoms of this disease that the writer has seen in Horal peas. Since the fungus was sharply limited in its development and became completely shut off after a time by the host responses, the complete sequence of symptoms could not be expected, particularly since, in this type of inoculation, the root system remained free from injury.

In spite of the indication of some degree of resistance to the fungus in the shoots of resistant and susceptible peas alike, these results agree with the writer's tests with toxic culture filtrates (2) in indicating that the

sharply defined varietal resistance is not a question of tolerance of toxic products of the wilt fungus, since the resistant and susceptible peas reacted essentially alike in the present case. Moreover, these results indicate that varietal resistance is expressed locally within the root system, probably during the attempted entry of the wilt fungus into the vascular system, as will be considered in the following paper. This is supported by May (3) who reports, in work published since the completion of this study, that the tomato-wilt Fusarium, introduced into the shoot of a resistant plant through a graft union with a susceptible plant, is able to advance and produce wilt symptoms.

SUMMARY

By inoculation of young pea plants with *Fusarium orthoceras* App. and Wr. var. *pisi* Linford through wounds in aerial parts, symptoms were obtained which partially simulated those typical of the pea-wilt disease. A higher percentage of infection was obtained in this way in the resistant variety, Horal, than in the susceptible variety, Badger, and the course of the resulting disease was similar in both varieties. These results are taken to indicate that the expression of varietal resistance to the pea-wilt disease is largely localized within the root system.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS,
HONOLULU, HAWAII.

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HYBRIDIZATION AND SEGREGATION IN THE OAT SMUTS

C. S. Holton[1]

INTRODUCTION

The discovery of physiologic specialization in smut fungi has stimulated investigations to determine whether or not physiologic forms arise by hybridization. Theoretically, new physiologic forms may arise either through hybridization between different forms or between different species, as well as by mutation. The writer has made studies to determine whether the two species of oat smuts, *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (K. and S.) Magn., hybridize.

REVIEW OF LITERATURE

Kniep (9) has shown that different species of smut will hybridize in culture to the extent that sporidia of opposite sex will fuse. Dickinson (3) has made histological studies which show that *Ustilago levis* and *U. hordei* (Pers.) K. and S. in combination produced infection in oats, whereas monosporidial lines alone produced no infection. However, he does not state that the infection referred to above resulted in the production of chlamydospores.

Kämmerling (8) obtained infection with crosses between monosporidial lines of opposite sex of *Ustilago longissima* (Sow.) Tul. and *U. longissima* var. *macrospora* Davis and found that similar hybrids occur in nature. Furthermore, Hanna and Popp (7) have obtained chlamydospore production by inoculating oat seedlings with crosses between monosporidal lines of *U. avenae* and *U. levis*.

Dickinson (4) has studied the nature of segregation within *Ustilago levis* and within *U. hordei*. He found that segregation for cultural characters was on a 2:2, 3:1, and 4:0 basis and that this segregation may take place in either of the "reduction divisions." Dickinson (2) found also that the segregation for sex factors in *U. levis* as well as in *U. hordei* was on a 2:2 basis, and Hanna and Popp (7) obtained similar results for *U. levis* and for *U. avenae*. That the segregation for sex factors is independent of the segregation of factors for cultural characteristics has also been demonstrated by Dickinson (4), and he was the first to show (5) that the nuclear division in which segregation occurs may be affected by alteration of the environmental conditions.

[1] The writer is greatly indebted to Dr. E. C. Stakman for suggesting the problem and to Dr. J. J. Christensen for many suggestions offered during the course of the investigations.

Stakman, Christensen, Eide, and Peturson (10) pointed out that if the numerous variants they obtained in *Ustilago zeae* (Beckm.) Ung. were not the results of mutations, they probably were due to some abnormal type of segregation. Christensen (10, Part II) obtained some indication of delayed segregation in certain self-fertile lines of *U. zeae*.

HYBRIDIZATION

In September, 1930, a large number of primary sporidia were isolated from the promycelia of germinating chlamydospores of *Ustilago avenae* and *U. levis*, and of this number 4 that were isolated from *U. avenae* and also 4 from *U. levis* were cultured on 1.3 per cent potato-dextrose agar. The 8 monosporidial lines thus secured, comprising 2 species, were paired in all possible combinations on nutrient-free agar and observed for fusions. The segregation for sex factors, as indicated by sporidial fusions, was found to be on a 2:2 basis with perfect interspecific fertility.

Pathogenicity tests were made by inoculating Anthony oats seedlings with intra- and interspecific crosses. Inoculations were also made with monosporidial lines and with combinations of monosporidial lines of similar sex, as indicated by the absence of sporidial fusions in culture. Plants that were not inoculated served as checks.

The inoculations were made in the following manner. The various monosporidial lines and crosses were cultured on nutrient-free agar in Petri dishes for 2 days. Ten cc. of sterile distilled water was then added to each culture, forming a suspension of the inoculum. Oat seedlings were placed in the inoculum and incubated at 18° to 20° C. for 48 hours, after which time they were transplanted to pots and incubated 6 days at the same temperature. The plants were then removed to the greenhouse where they grew to maturity. The results of the inoculations were obtained in November, 1930, and are summarized in table 1.

It is clearly evident from the data presented in table 1 that *Ustilago avenae* (spores echinulate) and *U. levis* (spores smooth) hybridize readily, as evidenced by the production of chlamydospores on the host. All combinations of monosporidial lines in which fusions were observed in culture produced chlamydospores on the host, the percentages of smutted panicles ranging from 23.8 to 70.5 in the interspecific crosses and from 18.8 to 72.7 in the intraspecific crosses. No smut was produced by inoculation with cultures of monosporidial lines alone or with combinations of monosporidial lines which did not fuse in culture.

Microscopic examinations revealed the fact that the interspecific hybrid chlamydospores were echinulate, while the intraspecific hybrid chlamydospores had markings characteristic of the species. Thus, echinulation is

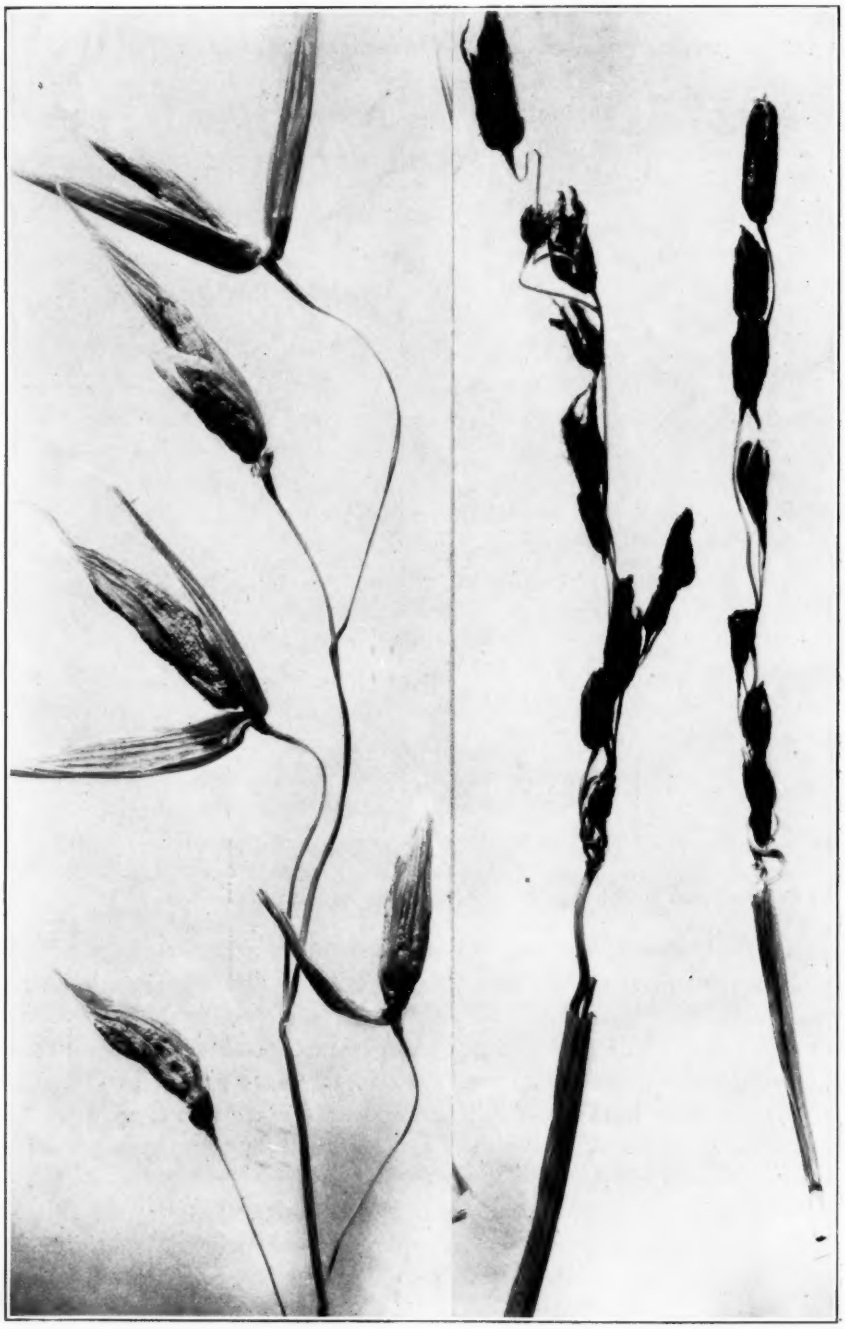
TABLE 1.—*The results of inoculating Anthony oats with intra- and interspecific crosses between monosporidial lines of Ustilago avenae and U. levis*

Lines and crosses	Number of seedlings inoculated	Number of panicles smutted	Percentage of smut
U. avenae			
1	20	0	0.0
2	25	0	0.0
3	32	0	0.0
4	30	0	0.0
1 × 3	22	16	72.7
2 × 4	28	5	18.8
2 × 3[a]	21	0	0.0
U. levis			
1	20	0	0.0
2	24	0	0.0
3	30	0	0.0
4	28	0	0.0
1 × 4	24	5	20.8
2 × 3	26	6	23.0
3 × 4[a]	27	0	0.0
U. avenae × *U. levis*			
1 × 3	34	24	70.5
2 × 1	24	12	50.0
4 × 3	21	5	23.8
4 × 4	21	12	27.1
Check	75	0	0.0

[a] Combinations in which sporidia did not fuse in culture.

apparently dominant over smoothness, in this case at least. Furthermore, the smutted panicles on the host were of the "loose" type in all cases of interspecific hybrid infection, although there was considerable variation in degree. Usually this type of injury can be attributed to *Ustilago avenae*, the species with echinulate spores. The intraspecific crosses produced the loose type of smut in the case of *U. avenae* and the covered type of smut in the case of *U. levis*. These results agree with those recently reported by Hanna and Popp (6).

Germination tests were made and it was found that the interspecific hybrid chlamydospores germinated and produced primary sporidia in a normal manner, but, when these sporidia were isolated, they failed to develop, except for occasional ones. More than 300 of these sporidia were isolated and only 5 cultural lines were obtained, representing both sexes.



A B

FIG. 1. Two types of oat smuts showing difference in color on the host.
A. Buff type. B. Normal, dark type.

Thus, in view of the fact that the primary sporidia isolated from germinating intraspecific hybrid chlamydospores developed normally in culture, it appears that some lethal factor or factors may be present and become effective in certain interspecific crosses. Whether this fact is generally true with all crosses between *Ustilago avenae* and *U. levis* has not been determined. Several different lines of *U. levis* have been crossed with the same line of *U. avenae* and in every case isolated sporidia failed to develop. It is entirely possible, if other lines of *U. evenae* were used, that the sporidia from some of the interspecific hybrid chlamydospores would develop normally.

For testing the pathogenicity of the interspecific hybrids 2 monosporidial lines of opposite sex have been crossed with each other and backcrossed with the parent lines. The back-cross with the *Ustilago avenae* parent produced infection (25 per cent) on Liberty Hulless oats and the chlamydospores were smooth, while the back-cross with the *U. levis* parent produced no infection on the same variety. The cross between the 2 monosporidial lines, originating from interspecific hybrid chlamydospores, produced infection (30 per cent) on Liberty Hulless oats and in this case the smut on the host was buff instead of black, the normal color for oat smuts. When examined microscopically, it was found that the individual chlamydospores were smooth and apparently colorless. They germinated normally and when sporidia were isolated from the promycelia these

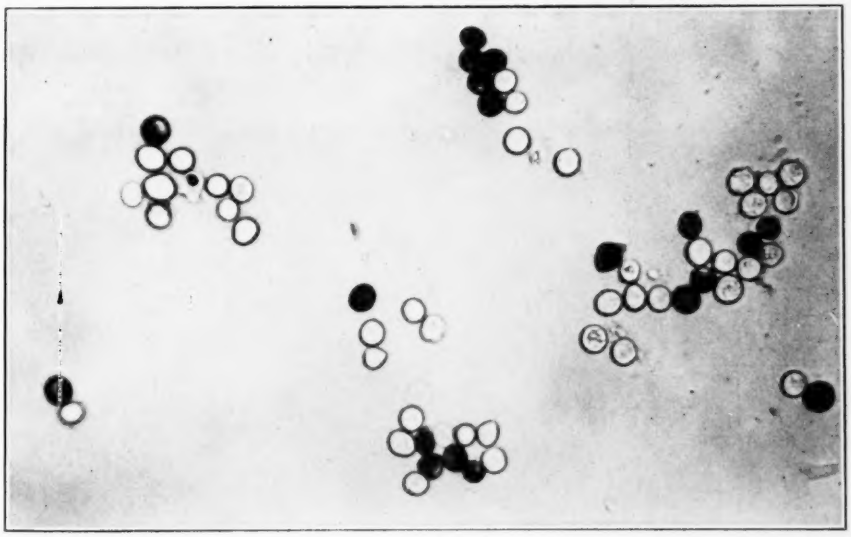


FIG. 2. Photomicrograph showing the contrast in color between the individual chlamydospores of the buff type of oat smut and the normal dark type.

sporidia reproduced normally in culture. The striking contrast between the buff and the normal type of oat smut on the host is shown in figure 1, while the contrast between the color of individual chlamydospores of the 2 types of smut is shown in figure 2. The pathogenicity of the buff type of smut has not been determined. Campagna (1) has described a buff type of loose smut of wheat occurring in nature.

SEGREGATION

The nature of segregation for sex factors and cultural characteristics has also been studied. All 4 primary sporidia have been isolated from a number of germinating chlamydospores by the method described by Hanna (6). As has already been stated, the segregation for sex factors was found to be on a 2:2 basis. Furthermore, all possible arrangements of these sexes on the promycelium have been found, indicating that reduction for sex may take place in either the first or second division of the reduction process.

Segregation of factors for cultural characteristics, such as color, topography, type of growth, and rate of growth, takes place in either division of the reduction process of *Ustilago avenae* and *U. levis* and segregation of these factors is independent of segregation for sex factors. Dickinson's work (4) has shown the same to be true for *U. levis*.

Successively produced sporidia were isolated from the same segment of the promycelium in order to determine whether or not further segregation occurs in the individual segments. If further segregation does not occur in the segments of the promycelium, then successively produced sporidia should develop lines that are identical in their cultural and sexual reactions.

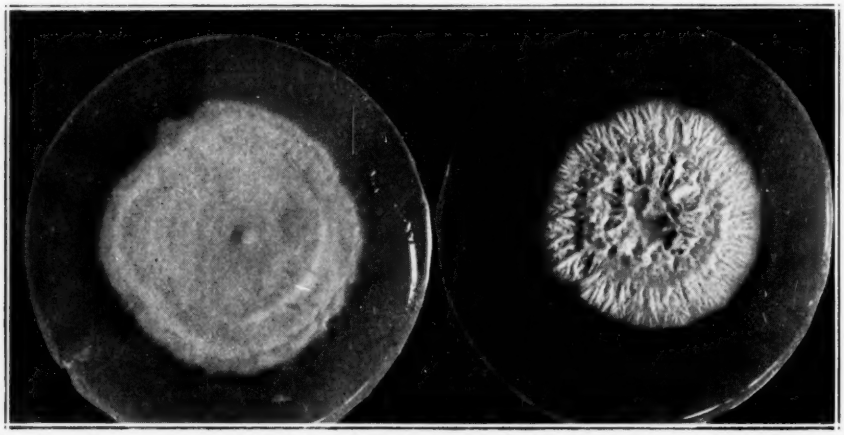


FIG. 3. Two monosporidial lines of *Ustilago avenae* obtained by isolating successive sporidia from the third segment of the promycelium of a germinating chlamydospore.

As many as 5 successive sporidia have been isolated from 1 segment and 2 to 3 successive ones from a number of other segments of the same promycelium and of different promycelia. Cultural comparisons of these isolations were made on 1.3 per cent potato-dextrose agar. Of the 5 monosporidial lines originating from the basal segment of 1 promycelium 4 were different in their cultural characteristics. In the majority of cases where 2 or 3 monosporidial lines originated from the same segment they were quite distinctly different in cultural characteristics. Typical cultural differences between lines originating from the same segment of the promycelium are

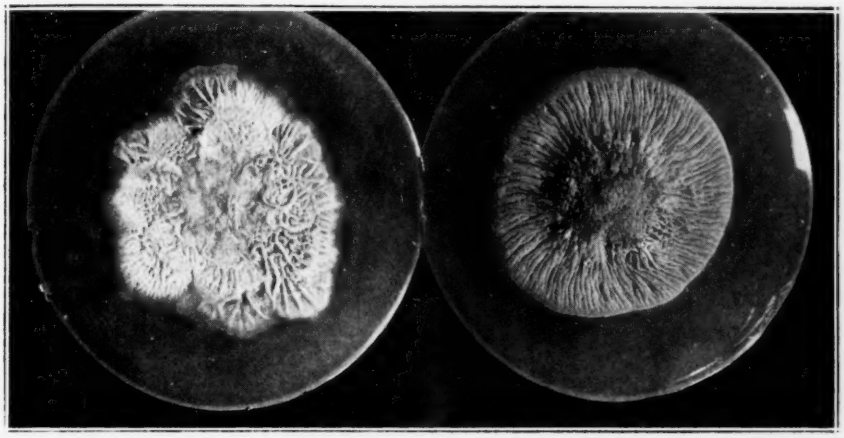


FIG. 4. Two monosporidial lines of *Ustilago levis* obtained by isolating successive sporidia from the second segment of the promycelium of a germinating chlamydospore.

clearly exhibited in figures 3 and 4. Thus, it seems quite evident that segregation for certain cultural characters is delayed beyond the second division of reduction division in *Ustilago avenae* and *U. levis*. All monosporidial lines originating from the same segment of the promycelium were of the same sex. Therefore, it appears that segregation for sex factors usually takes place in either the first or second division of reduction division and not in subsequent divisions as it does for other characters.

SUMMARY

1. *Ustilago avenae* and *U. levis* are perfectly interfertile. Monosporidial lines of opposite sex fuse in culture and also produce smut on the host, regardless of whether crosses are made inter- or intraspecifically.

2. Sporidial fusions do not occur within a monosporidial line of either species and neither do they occur in intra- and interspecific combinations of monosporidial lines of similar sex. Furthermore, when such combinations are used for inoculation, no smut is produced on the host.

3. Intraspecific crosses produced the loose type of smut in the case of *Ustilago avenae* and the covered type in the case of *U. levis* while the smut produced by the interspecific crosses was of the loose type.

4. Intraspecific hybrid chlamydospore markings were characteristic of the species, but the interspecific hybrid chlamydospores were echinulate.

5. Primary sporidia isolated from the promycelia of germinating interspecific hybrid chlamydospores would not develop in culture, with rare exceptions. However, primary sporidia isolated from the promycelia of germinating intraspecific hybrid chlamydospores developed in a normal manner.

6. A cross between two monosporidial lines that originated from interspecific hybrid chlamydospores produced a buff type of smut which is apparently a new and previously undescribed type. The chlamydospores of this smut were smooth and apparently colorless, in contrast to the dark brown chlamydospores of the common type of oat smut.

7. Segregation for sex factors in *Ustilago avenae* and *U. levis* was found to be on a 2:2 basis. There is strong evidence that a delayed segregation of factors for certain cultural characteristics occurs in *U. avenae* and *U. levis* since monosporidial lines originating from the same segment of the promycelium often exhibit striking differences in cultural characteristics.

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